ejpmr, 2019,6(1), 111-115

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF THE ANTIFUNGAL ACTIVITY OF HYDRO-ALCOHOLIC EXTRACTS OF LEAVES OF THREE PLANTS ON MUSHROOMS OF RICE, MAIZE, COWPEAS AND PEANUT SEEDS

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Article Received on 07/11/2018 **Article Revised on 28/11/2018** **Article Accepted on 19/12/2018**

SUMMARY

To help find alternative control methods against seed-borne microorganisms, a study was undertaken to evaluate the antifungal activity of three plant extracts on rice, maize, cowpea and groundnut seeds. . To this end, the efficacy of the hydroalcoholic extracts of Hyptis suaveolens, Western Anacardium and Azadirachta indica was measured on the fungi Aspergillus flavus, Aspergillus niger, Alternaria padwickii and Fusarium moniliforme of the studied seeds. Sanitary tests of the infected seeds were carried out by the paper method after being soaked in hydroalcoholic extracts. Then, the effect of these extracts on the germination rate of the treated seeds was evaluated by the method of between papers. This study shows that the three extracts reduce or inhibit differently the infection rate of fungi found on seeds.

KEYWORDS: antifungal activity, hydroalcoholic extracts, seeds, fungi.

INTRODUCTION

Seeds play a vital role in crop production, agriculture and world food. Seed quality determines the quality of productivity, although rainfall, agronomic practices, soil fertility and pest control are also essential (Turner, 2010). However, the seed is subjected to multiple aggressions which influence the different phases of its development which are the germination, the development of the root system and the growth of the aerial parts (Amine *et al.*, 2017). Several pests are causing these attacks such as insects, bacteria, viruses, nematodes and fungi (Parveen *et al.*, 2014). Fungi not only degrade seed quality, but also transmit certain diseases that cause lower yields and the depreciation of agricultural products (Orsot *et al.*, 2015).

To control seed fungi, several methods are used. However, the one that remains the most effective is the use of chemical fungicides. Repeated use of these chemicals may induce resistance of these microorganisms to fungicides and have adverse environmental consequences (Corbaz, 1990). Awareness of the environmental cost of using chemicals and the fear of consumers of the potential risks of pesticide residues to human health are generating growing interest in other control alternatives (El Guilli *et al*., 2009). Thus, the exploitation of the fungicidal and pesticidal properties of plant extracts is more and more considered. The general objective of this study is to evaluate the antifungal activity of the hydro-alcoholic extracts of three plants on rice seed fungi, maize, cowpea and peanut.

MATERIAL AND METHODS

Material: Arica 4 rice seeds, espoir variety corn, Nafi cowpeas and RMP12 groundnuts are sourced from the National Seed Laboratory (LANASEM, Cote d'Ivoire). The hydro-alcoholic extracts of the leaves harvested in the Korhogo region (Cote d'Ivoire) of *Hyptis suaveolens*, Azadirachta indica, and *Anacardium occidentale*.

Methods: Fresh leaves of *Hyptis suaveolens*, *Azadirachta indica*, and *Anacardium occidentale* were milled using a Moulinex-type grinder. The ground material from one (1) kilogram of leaves was macerated in 10 mL of hydro alcoholic solution for 24 hours with stirring. The mixture obtained was sieved then filtered through a cloth and stored in a refrigerator.

Seed treatment: 400 grains of corn and cowpea were soaked in 80 mL of hydro alcoholic solution (10 %) and those of rice and peanut were soaked in 50 and 100 mL for 24 hours, respectively. The control consisted of untreated seeds and those treated with a fungicide. The variation in grain immersion volumes depends on the size of the seeds.

Seed health analysis: The health status of the seeds was determined by the blotting paper method (Mathur et

kongsdal, 2003). This method involves placing the seeds (25) equidistant on blotting paper moistened with distilled water sterilized in 9cm petri dishes. These boxes thus seeded are incubated for seven days at 20 ° C. under an alternating cycle of 12 hours of light and 12 hours of darkness. Petri dishes are read using a binocular loupe to observe phytopathogenic fungal germs on seeds and to identify them according to their morphological, cultural and biochemical characteristics.

Seed germination test: The method of Between papers was used (Kameswara *et al.*, 2006). It consists of arranging twenty-five (25) grains of seed equidistant from each other between two sheets of blotting paper soaked with sterile distilled water in 9cm petri dishes. The petri dishes are then incubated in a germinator under an alternating cycle of 8 h light at 20 ° C and 16 h at 30 ° C per day. The evaluation of the germination rate consisted in counting the germinated grains, the hard grains as well as the dead grains. The results of this count were expressed as a percentage and the germination rate corresponds to the percentage of sprouted seeds.

RESULTS AND DISCUSSIONS

Seed health analysis: Several fungi were detected on the tested seeds. Rice seeds were infected only by *Alternaria padwickii (A. padwickii)* at a rate of 2%. %. The maize sample was contaminated with *Aspergillus flavus (A. flavus), Aspergillus niger (A. niger), Alternaria padwickii (A. padwickii)* and *Fusarium moniliforme (F. moniliforme)* with respective infection rates of 5%, 8%, 6% and 2.5%. Cowpea seed showed an infection rate of *A. flavus* of 30%, *F. moniliforme* of 17%, A. padwickii of 10.5% and *F. culmorum* of 7%. Peanut seeds were contaminated with *A. padwickii* 75% and *A. flavus* 16% **(figure 1).**

Effect of hydro-alcoholic extracts on the infection rate of rice seeds

Extracts of *Hyptis suaveolens, Azadirachta indica*, and *Anacardium occidentale* were all effective against *A. padwickii* germ detected on rice seeds (**table 1)**

Effect of hydro-alcoholic extracts on the infection rate of corn seeds

The three hydro-alcoholic extracts of plants have various activities on the *A. niger* microorganism. Indeed, extracts of *Hyptis suaveolens* and *Azadirachta indica* have no effect on the growth of this germ while that of *Anacardium occidentale* reduces its development. These hydro-alcoholic solutions completely inhibit *A. padwickii* and have no influence on the normal growth of *A. flavus* and *F. moniliforme* **(table 2).**

Effect of hydro-alcoholic extracts on the infection rate of cowpea seeds: Cowpea seed treated with extracts of *Hyptis suaveolens, Azadirachta indica,* and *Anacardium occidentale* still retain their level of infection with *A. flavus* and *F. moniliforme*. This treatment destroyed the growth of *A. padwickii*. The germ of *F. culmorum* was inhibited by extracts of *Hyptis suaveolens* and *Azadirachta indica* (**table 3).**

Effect of hydro-alcoholic extracts on peanut seeds infection rate: The hydroalcoholic extracts of *Hyptis suaveolens,Aazadirachta indica*, and *Anacardium occidentale* were all effective against the *A. padwickii* germ and ineffective against the *A. flavus* fungus **(table 4).**

Effect of treatments on germination of rice seeds

Untreated rice seed has a germination rate of 85-95%. These seeds treated with hydro-alcoholic extracts gave germination rates of 86.5% with *Hyptis suaveolens*, 94% with *Azadirachta indica*, and 95% with *Anacardium occidentale* **(figure 2)**.

Effect of treatments on germination of corn seeds

Untreated maize seed has a germination rate of 89-96%. The treatment of these seeds with the hydro-alcoholic extracts of the plants revealed a considerable drop in the germination rate. For example, extracts of *Hyptis suaveolens*, *Azadirachta indica*, and *Anacardium occidentale* showed low germination percentages of 4%, 1%, and 23.5%, respectively **(figure 3)**.

Effect of treatments on seed germination of cowpea

Untreated cowpea seed has a germination rate of 30-40%. No seed germinated after treatment with extracts of *Hyptis suaveolens* and *Azadirachta indica*. However, with *Anacardium occidentale* extracts, the germination rate was 5% **(figure 4)**.

Effect of treatments on germination of peanut seeds

Untreated peanut seeds have a germination rate of 66-75%. The percentage of germination was variable with the extracts of the different plants. Solutions of *Hyptis suaveolens, Azadirachta indica* reduced the germination rate of peanut seeds by 10% and 17%, respectively, while that of *Anacardium occidentale* decreased by 68% **(figure 5).**

Table. 1: Effect of hydro-alcoholic extracts on the infection rate of rice seeds.

	Hydro-alcoholic extracts of plants		
Germ	***A. occidentale***	***A. indica***	***H. suaveolens***
A. padwickii	-	-	-

(+): infected (-): not infected

Table. 2: Effect of hydro-alcoholic extracts on the infection rate of corn seeds.

	Hydro-alcoholic extracts of plants		
Germs	***A. occidentale***	***A. indica***	***H. suaveolens***
A. padwickii	-	-	-
A.flavus	+	+	+
A.niger	+	+	+
F. moniliforme	+	+	+

(+): infected (-): not infected

Table. 3: Effect of hydro-alcoholic extracts on the infection rate of cowpea seeds.

	Hydro-alcoholic extracts of plants		
Germs	***A. occidentale***	***A. indica***	***H. suaveolens***
A. padwickii	-	-	-
A. flavus	+	+	+
F. culmorum	+	-	-
F. moniliforme	+	+	+

(+): infected (-): not infected

Table. 4: Effect of hydro-alcoholic extracts on the infection rate of peanut seeds

	Hydro-alcoholic extracts of plants		
Germs	***A. occidentale***	***A. indica***	***H. suaveolens***
A. padwickii	-	-	-
A. flavus	+	+	+

(+): infected (-): not infected

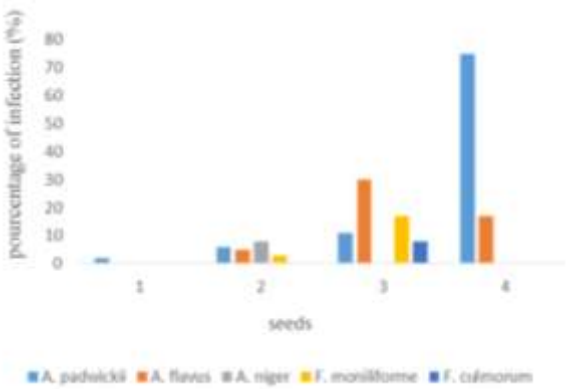

Figure. 1: Health status of different seeds.
1: rice 2: corn 3: cowpea 4: peanut

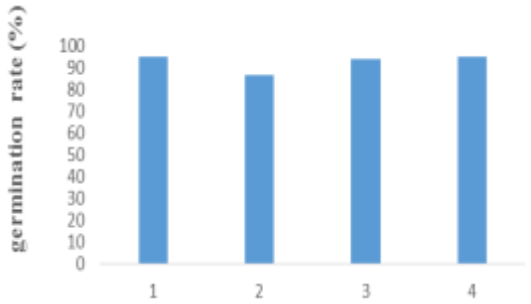

Figure. 2: Germination rate of rice seeds treated with plant extracts.
1: germination rate of untreated rice (control).
2: germination rate of treated rice with H. suaveolens.
3: germination rate of treated rice with A. indica.
4: germination rate of treated rice with A. occidentale.

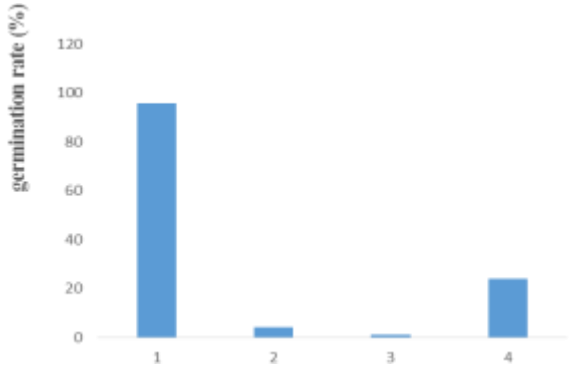

Figure. 3: Germination rate of corn seeds treated with plant extracts.
1: germination rate of untreated corn (control).
2: germination rate of treated corn with H. suaveolens.
3: germination rate of treated corn with A. indica.
4: germination rate of treated corn with A. occidentale.

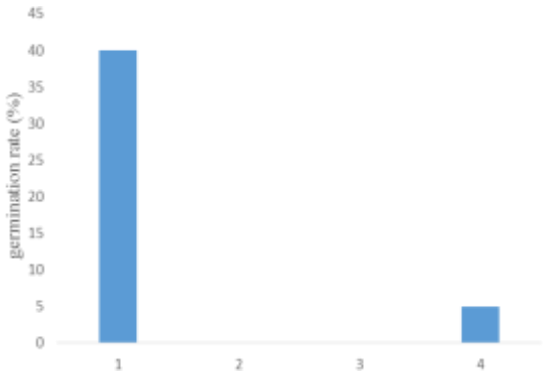

Figure. 4: Germination rate of cowpea seeds treated with plant extracts.

1: germination rate of untreated cowpea (control).
2: germination rate of treated cowpea with H. suaveolens.
3: germination rate of treated cowpea with A. indica.
4: germination rate of treated cowpea with A. occidentale.

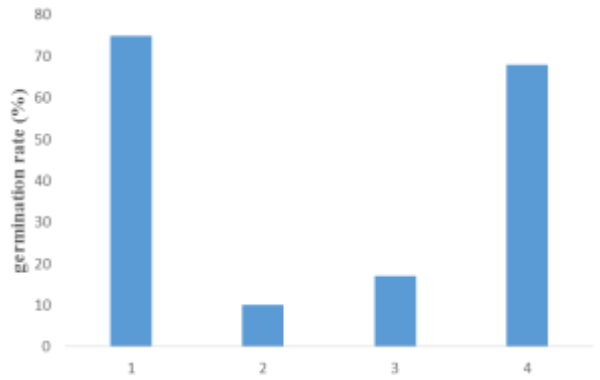

Figure. 5: Germination rate of peanut seeds treated with plant extracts.
1: germination rate of untreated peanut (control).
2: germination rate of treated peanut with H. suaveolens.
3: germination rate of treated peanut with A. indica.
4: germination rate of treated peanut with A. occidentale.

DISCUSSIONS

The result of the health status of the seeds revealed the presence of several microorganisms such as fungi in rice, maize, cowpea and peanut seeds. The identified fungi were of the genus *Aspergillus*, *Alternaria* and *Fusarium*. These results are consistent with those of several authors who also detected various fungi in seeds after health analysis (Kanoun *et al.*, 2014; Ilboudo *et al.*, 2016). Thus, *Phytophthora colocasiae* was found in the seeds of tarot (Tsopmbeng *et al.*, 2014), *A. flavus* and *A. niger* were highlighted in maize seeds in Burkina Faso (Dao, 2013). The presence of these fungi in the analyzed samples is due to infections during harvest or seed storage.

The hydro-alcoholic extracts tested had varied effects on the different fungi found on the seeds. Some have been effective while others have been without action on these microorganisms. Thus, the hydroalcoholic extracts of the three plants completely inhibited the *Alternaria padwickii* germ, whereas *A. flavus*, *F. moniliforme*, and *A. niger* mushrooms developed in their presence. However, the pathogen *F. culmorum* was inhibited by *H. suaveolens* and *A. indica* solutions. This plant inhibitory property has been highlighted by several authors (Tiendrébeogo, 2011; Bonzi, **2013,** Benmeddour et Fenni, 2018).

The study of the state of health of seeds is of paramount importance for the development of agriculture. In fact, it is necessary to ensure a good sanitary status of the seeds because diseases already present on these seeds could lead to the gradual development of diseases in the field and reduce the commercial value of crops or imported seed lots could introduce diseases into regions where they were not present (FAO, 2011). Seeds are also crucial to face the challenges of food insecurity. To achieve food security, farmers depend on quality seeds and varieties appropriate to their needs.

The different seeds studied are of major importance in food security in African countries and particularly in Côte d'Ivoire. Indeed, rice (rainfed rice, lowland rice and irrigated rice) is the first food consumed and the most widely grown cereal in Côte d'Ivoire (Kouadio, 2010). Maize, Ivory Coast's fifth-largest food production, has two production systems compared to the main agro-ecological zones, namely the forest zone and the savannah zone. It is used in human and animal food (Yeo, 2011). Cowpea is grown in the Ivory Coast mainly in the savannah regions for its seeds consumed as a dry vegetable in combination with millet, sorghum, maize and yam because it enriches the soil by fixing atmospheric nitrogen (konaté *et al.*, 2012). Peanuts are grown in all the agro-ecological zones of the Ivorian territory (savary *et al.*, 1987) and is of great importance for these by-products such as oil and cakes used in food and feed.

CONCLUSION

The results of this work show that rice, maize, cowpea and peanut seeds are infected with fungi (*Aspergillus*, *Alternaria* and *Fusarium*) at different rates. The treatment of these seeds with the hydro alcoholic extracts of *Hyptis suaveolens*, *Azadirachta indica* and *Anacardium occidentale* demonstrated the antifungal activity of these extracts. However, the antifungal activity of one extract varies from one microorganism to another and no extract was effective against all germs of different seeds. Nevertheless, the efficiency of certain extracts is of paramount importance for agriculture, particularly in developing countries, where the majority of farmers lack the means to acquire quality seeds.

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