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Efficacy of Three Plant Extracts Against Three Fungi that Cause Papaya (*Carica Papaya L.*) Fruit Rot

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Abstract: ***In Côte d'Ivoire, the control of chemical fungal diseases poses residue problems on the papaya. Thus, the use of plant extracts is proving to be a recommended alternative. The objective of this work is to contribute to the fight against postharvest fungal rot of papayas through the use of plant extracts. The effects of aqueous extracts of seeds of Azadirachta indica, Ricinus communis and Jatropha curcas at concentration of 50, 125 and 250 mg / ml were determined in three fungal genera, namely Fusarium, Phytophthora and Colletotrichum isolated from papaya. Aqueous extracts of Jatropha curcas and Ricinus communis completely inhibited mycelial growth of the three fungal genera at a concentration of 250 mg / ml. The papayas remained rot-free in the presence of seed extract of Ricinus communis and Jatropha curcas at the same concentration. However, the toxicity of these extracts should be evaluated.***

Keywords: Papaya, Rot, fungi, control, plant extract

1. Introduction

Papaya (*Carica papaya* L., Caricaceae) is a plant grown in tropical and subtropical regions for its fruit [1]. The fruit of papaya is eaten for its richness in vitamins and for its therapeutic effects [2]. Indeed papaya provides sources of income and important vitamins for food especially vitamins A and C. World production was estimated at 12.67 million tons in 2014. India is the world's leading producer with 5.63 million tons, followed by Brazil; 1.60 million tons [3]. In Africa, Nigeria is the leading producer with a production of 850 000 tons [3]. With 14,028 tons, Côte d'Ivoire is the eighth largest African producer of papaya. In the last five years, papaya production in Côte d'Ivoire has increased significantly from 11,626 tons to 14,028 tonnes [3]. However, this increase in production volume is mainly due to an extension of production area, contrary to the production per hectare which is declining overall [3].

Indeed, the cultivation of papaya is threatened by pests and diseases [4]. One of the causes of yield declines is the development of fungal diseases. Thus, papaya is faced with the problems of rotting fruit. Fungal pathogens are usually responsible for these rots. As a result, post-harvest rots reduce the yield and quality of the papaya market.

In addition, several control methods exist to reduce post-harvest papaya diseases. These include physical treatments [5], use of antimicrobial substances [6], organic and inorganic compounds [7]. Chemical control is the most used [8]. However, chemical control methods against these post-harvest fruit diseases pose enormous problems of residues and toxicity to consumers [9]. In addition, according to [10], the unrestrained use of fungicides causes resistance to these fungicides in the population of post-harvest pathogens.

Chemical control methods with residue problems tend to be abandoned in favor of the use of plant extracts. Plant extracts have the advantage of being not only available cheaply for farmers, but also non-toxic and biodegradable [11]. All parts of the plant *Azadirachta indica* can be used in pest control. [12]. Its pest management has been widely studied and proved effective against several diseases and crop pests [13, 14]. Thus tests carried out by [15] showed the antifungal activity of *Azadirachta indica* extracts on the mycelial growth of *Fusarium oxysporium* and *Rhizoctonia solani*, fungi that cause enormous losses in tomato production. Similarly, [16] showed the antifungal properties of the extract of *R. communis* leaves against *Alternaria solani* causing the alternaria of tomato. According to [17], the chloroform and hexane extract of *Jatropha curcas* seeds can be used as a natural phytochemical against certain fungal and bacterial plant pathogens. [18] also demonstrated *the in vitro* and *in vivo* efficacy of *Jatropha curcas* and *Ricinus communis* seeds on *Fusarium verticilliodes* and *Aspergillus flavus*, which causes yam tubers to decay.

The purpose of this study was to determine the efficacy of three plant extracts against three fungi that cause post-harvest papaya fruit rot.

2. Material and Methods

Plant material

The plant material used consists of apparently healthy variety of solo papayas and seeds of neem (*Azadirachta indica*), jatropha (*Jatropha curcas*) and castor (*Ricinus communis*). Neem seeds were collected at Assabou (Yamoussoukro district). Jatropha seeds were collected in N'Gattakro locality (Yamoussoukro) while castor seeds were collected at Banco 1 (Yopougon commune).

Methods

Preparation of aqueous extracts of seeds Harvested neem seeds were pulped. Seeds of castor and *J. curcas* have been cleared of their pods. The seeds of *A. indica*, castor and *J. curcas* were dried separately in the laboratory for 30 days. Then they were shelled. For the preparation of aqueous extracts, 50 g of seeds of *A. indica*, castor and *J. curcas* were washed separately with tap water and spread on blotting

paper at laboratory temperature (27 ± 2° C) for one hour drying. They were then disinfected with 8° of sodium hypochlorite (2.4 % active chlorine) at 10 % for 5 min, then rinsed three times with tap water and dried in the shade for 2 h. The seeds were then ground separately in a laboratory mortar and then placed in 100 ml of sterile distilled water each giving a stock concentration of 500 mg / ml. These mixtures were collected in the jars and left to macerate for 48 h in the absence of light according to the method of [19]. Each mixture was then filtered through two folds of sterile muslin to remove coarse elements. The extract obtained was sterilized by filtration on sterile hydrophilic cotton according to the method of [20].

Preparation of the amended culture medium of plant extract

The amended PDA extract medium was used to perform the *in vitro* efficacy test. For the preparation of 1L of PDA medium, 20 g of Agar was dissolved in 1 L of potato extract. The potato extract was obtained after boiling 200 g of raw potato for 30 min. The potato was then crushed and filtered to obtain the extract [21]. Then 20 g of glucose were added to the whole [21]. After sterilization in an autoclave at 120 ° C., at 1 bar and for 30 min, 135; 112.5 and 75 ml of supercooled PDA medium respectively received volumes 15; 37.5 and 75 ml of pre-prepared extracts in the proportions 10 %, 25% and 50 %, which gave the respective concentrations of 50, 125 and 250 mg / ml for a Petri dish. After homogenization, each medium was distributed in 90 mm Petri dishes in diameter at a rate of 10 ml per Petri dish and then allowed to solidify. Thus, for each plant extract these three concentrations were observed.

***In vitro* efficacy test of plant extracts on mycelial growth**

The three fungal genera used were subcultured on PDA media supplemented with different concentrations of extracts. A fungal inoculum 5 mm in diameter was taken from pure cultures aged 7 days on PDA medium. The inoculum was placed in the center of the Petri dishes. Fifteen Petri dishes were used for each concentration, ie 45 Petri dishes for each extract. Fifteen other Petri dishes containing the PDA medium without extracts served as a control. These Petri dish isolates were then incubated at room temperature (27 ± 2 ° C) until complete colonization by the fungal genera of the surface of the PDA medium in the control dishes.

***In vivo* efficacy test of plant extracts on mycelial growth**

In vivo tests were performed according to the modified [22] method. Sixty mature fruits of the solo variety were previously disinfected in a mixture of 10 % sodium hypochlorite solutions (2.4 % active chlorine) and 70 % alcohol followed by three rinses with sterile distilled water. Then, wounds 5 mm in diameter were made on the fruits using a scalpel blade. Fifteen fruits were immersed for 5 min in each of the extracts at the concentration having exhibited fungitoxic activity and then dried. Finally, a fungal inoculum of 5 mm in diameter was removed from 7-day-old cultures on PDA medium (three fungal genera responsible for papaya rot) and deposited in wounds. Inocula were covered with hydrophilic cotton soaked in water to maintain moisture. For each fruit, an inoculum of each fungal genus was deposited on the wounds performed. Five fruits were used per fungal genus and 15 fruits were used per extract. Fifteen inoculated fruits that had not been previously treated constituted the controls. The fruits treated with the different extracts were distributed separately in plastic pots and closed, in order to maintain a high relative humidity. Pots were then placed in bins and incubated in the dark at ambient temperature of the laboratory (27 ± 2 ° C). Rots were evaluated 7 days after inoculation. The experiment was repeated three times.

Parameters evaluated

Mycelial growth of fungal genera

The cultural characteristics such as the appearance and the evolution of the mycelial colonies of the strains subcultured on the PDA amended media of plant extract have been described according to the type of extract and the concentration. Daily growth of each fungal genus was determined by measuring the diameter of the mycelium along the two axes previously plotted at the back of each Petri dish using a graduated ruler. The rate of inhibition of mycelial growth of the isolates by seed extracts was calculated after 10 days of incubation according to the formula of [23] :

$$I\,(\%) = \frac{C-T}{C} \times 100$$

I: Rate of inhibition of mycelial growth.
C: Radial growth (mm) of the control
T: Radial growth (mm) of the treatment

In vivo efficacy of the plant extracts

The measurement of the lesion diameters was made along two perpendicular axes on the fruits, considering that the lesions are circular. The rate of inhibition of the lesion caused by the seed extracts was calculated according to the formula of [22].

$$I\,(\%) = \frac{(Do-D)}{Do} X\,100$$

I: Inhibition rate of the lesion
Do: control lesion diameter
D: lesion diameter of the fruit treated with the extract

Statistical analyzes

The data obtained were subjected to a one-way classification criterion of the variance analysis (ANOVA 1). The non-parametric Kruskal-Wallis rank test was performed for the evaluation of the isolation frequency of fungal genera; fungal decay volume after inoculation in the pathogenicity test and inhibition rates of mycelial growth, spore germ tube elongation and rot of treated papayas for each fungal genus. These analyzes were carried out thanks to the software Statistica version 7.1. The Mann Whitney U test was used to classify the means for the determination of homogeneous groups, in case of difference at the threshold $\alpha = 0.05$.

3. Results

Inhibition of mycelial growth of fungal genera by seed extracts

***Azadirachta indica* extract**

Inhibition of mycelial growth of *Fusarium*, *Phytophthora*, and *Colletotrichum* varied with different concentrations. It

was higher with increasing concentrations of the extract (Table 1). For fungal genera, inhibition percentages were statistically higher at 125 and 250 mg / ml. Total inhibition of mycelial growth of *Phytophthora* was observed at the concentration of 250 mg / ml.

***Ricinus communis* extract**

The sensitivity of the fungal genera of *Fusarium*, *Phytophthora* and *Colletotrichum* varied according to the different concentrations. The fungal genera were more sensitive according to increasing concentrations of the extract (Table 2). For the fungal genera, inhibition percentages were statistically higher at 125 and 250 mg / ml. Total inhibition of mycelial growth of the genus *Phytophthora* was observed at the concentration of 250 mg / ml.

***Jatropha curcas* extract**

The inhibition of mycelial growth of different fungal genera was variable in the presence of different concentrations of the extract. It was higher in increasing concentrations (Table 3). For the three fungal genera, inhibition percentages were statistically higher at 125 and 250 mg / ml. Total inhibition of mycelial growth was observed at the concentration of 125 mg / ml for *Colletotrichum* and for all three fungal genera (*Fusarium*, *Phytophthora* and *Colletotrichum*) at 250 mg / ml.

4. Discussion

The antifungal activity of the three extracts of *Azadirachta indica*, *Ricinus communis* and *Jatropha curcas* were evaluated on three fungal genera : *Fusarium*, *Phytophthora* and *Colletotrichum*. The aqueous extracts of the seeds of these three plants showed their ability to inhibit *in vitro* mycelial growth of the three fungal genera used. These extracts also inhibited rots of papayas in the presence of these fungal genera. These results obtained are due to the photochemical composition of the various extracts. In the presence of the neem extract, the three fungal genera were inhibited at the three concentrations used on PDA medium supplemented with extracts. Similar results were obtained by [24] with a range of concentrations of ethanolic, hexane and methanolic extracts of *Azadirachta indica* seeds varying from 10 to 40 g / ml. Indeed, these authors evaluated *in vitro* the inhibition of the growth of *Alternaria solani*, *Fusarium oxysporium*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* by the extract of neem seeds.

This inhibition of mycelial growth of fungal genera could be due to the simultaneous action of neem chemical compounds. Azadirachtin, an active substance with nimbidine, solanine, melantriol and triterpenoids, are found in these aqueous extracts by [25] and [26]. The inhibition of rots by the aqueous extract of neem was evaluated at more than 87.20%. These results are in agreement with those of [12] who have highlighted the effectiveness of the oil extract of neem seeds. This substance applied at the rate of 10 l / ha was able to significantly reduce the incidence of brown rot of cocoa pods.

Aqueous extracts of castor and jatropha inhibited different fungi with *in vitro* and *in vivo* tests. Indeed, the mycelial growth of these fungal genera was reduced with the three concentrations used. These results are in agreement with those obtained by [27] which showed the antifungal activity of methanol extracts of pericarp and leaf of *Jatropha curcas* on *Trametes versicolor*, *Gleophyllum trabeum* and *Chaetomium globosum*. Indeed, these authors used a series of concentrations ranging from 50, 100, 250, 500 and 1000 µg / ml. The browning of papayas during the *in vivo* test is due to the toxicity of ricin with a high amount contained in castor seeds. In this study, inhibition rates on the highest fungal genera were observed at high extract concentrations in the medium for the different extracts. This indicates that the extracts develop a good antifungal activity when the active substances are more concentrated in the culture medium.

The aqueous extract of *Jatropha curcas* had a higher antifungal activity than the aqueous castor extract which also had a higher antifungal activity than that of *Azadirachta indica*.

This variation could be attributed on the one hand to the drying conditions of the seeds and on the other hand to the extrinsic factors of each plant.

5. Conclusion

The results of this study indicate that the aqueous extracts of *Azadirachta indica*, *Rcinus communis* and *Jatropha curas* on mycelial growth and the elongation of the spore germ tube caused up to 100 % inhibition. This inhibition was high for all fungal genera tested at the concentration of 250 mg / ml. The three extracts are fungitoxic for all fungal strains. These extracts were effective at a concentration of 250 mg / ml for papaya tests. Aqueous extracts could therefore be an alternative to chemical fungicides in the fight against rot of papaya fruits.

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Appendices

Table 1: Rate of inhibition of mycelial growth of fungal genera as a function of the concentration of the extract of *Azadirachtha indica* ten days after incubation

Concentration (mg/ml)	*Average rate of inhibition (%)*		
	Fusarium	*Phytophthora*	*Colletotrichum*
50	33.00 ± 4.88^{b}	18.50 ± 3.31^{b}	57.75 ± 2.92^{b}
125	57.25 ± 2.15^{a}	75.12 ± 3.43^{a}	79.62 ± 8.50^{a}
250	66.12 ± 5.25^{a}	100.0 ± 0.00^{a}	94.12 ± 2.44^{a}
H	10.22	12.96	9.17
P	0.006	0.001	0.01

The assigned numbers of the different letters are significantly different according to the Mann-Whitney U test at the threshold of 5 %

Table 2: Rate of inhibition of mycelial growth of fungal genera as a function of the concentration of the extract of *Ricinus communis* ten days after incubation

Concentration (mg/mL)	*Average rate of inhibition (%)*		
	Fusarium	*Phytophthora*	*Colletotrichum*
50	52.25 ± 4.64^{b}	76.62 ± 5.70^{b}	57.75 ± 2.92^{b}
125	69.50 ± 1.86^{a}	99.12 ± 1.14^{a}	79.62 ± 8.50^{a}
250	73.87 ± 1.88^{a}	100.0 ± 0.00^{a}	94.12 ± 2.44^{a}
H	9.62	10.55	10.47
P	0.008	0.005	0.005

The assigned numbers of the different letters are significantly different according to the Mann-Whitney U test at the threshold of 5 %

Table 3: Rate of inhibition of mycelial growth of fungal genera as a function of the concentration of the extract of *Jatropha curcas* ten days after incubation

Concentration (mg/mL)	*Average rate of inhibition (%)*		
	Fusarium	*Phytophthora*	*Colletotrichum*
50	51.00 ± 1.40^{c}	77.37 ± 3.36^{b}	89.87 ± 0.30^{b}
125	69.25 ± 6.00^{b}	88.62 ± 4.86^{b}	100.0 ± 0.00^{a}
250	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
H	12.96	8.43	13.42
P	0.001	0.01	0.001

The assigned numbers of the different letters are significantly different according to the Mann-Whitney U test at the threshold of 5 %

Table 4: Rate of inhibition of rots of each fungal genus according to the type of extract

Extracts	*Average rate of inhibition (%)*		
	Fusarium	*Phytophthora*	*Colletotrichum*
Azadirachtha indica	87.20 ± 5.27^{b}	100.0 ± 0.00^{a}	93.60 ± 3.70^{a}
Ricinus communis	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
Jatropha curcas	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
H	6.92	-	2.98
P	0.03	-	0.08

The assigned numbers of the different letters are significantly different according to the Mann-Whitney U test at the threshold of 5 %

Figure 1 : Effect of aqueous extracts of plants on rots seven days after inoculation of papayas at a concentration of 250 mg / ml

A : *Colletotrichum*; B : *Fusarium*; C : *Phytophthora*; Control : untreated with extracts

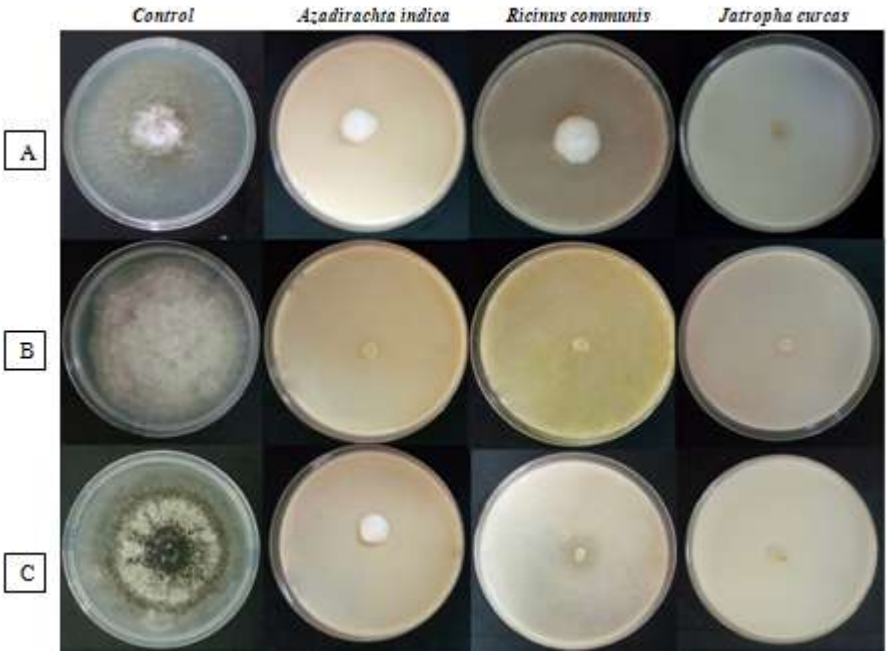

Figure 2 : Effect of aqueous extracts of plants on mycelial growth of fungal genera ten days after incubation on modified PDA medium of different extracts at a concentration 250 mg / ml
A : *Fusarium*; B : *Phytophthora*; C : *Colletotrichum*; Control : untreated with extracts