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Biochemical Resistance Mechanism Study of *Jatropha curcas* (Euphorbiaceae) Against *Lasiodiplodia theobramae*, a Leaf Blight and Necrosis Agent

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Abstract

This study was initiated in order to understand the biochemical mechanisms involved in the resistance of *J. curcas* to *Lasiodiplodia theobromae.* Artificial inoculations were done on young seedlings leaves of 8 *J. curcas* local accessions from Burkina Faso including 4 resistant and 4 susceptible. Two extractions were made for the evaluation of each biochemical parameter: one on the 1st day before and the second on the 7th day after inoculation. The study revealed that the response of *J curcas* to *L. threobromae* infection is depending to the resistance or the susceptibility of accessions. It showed an increase in the content of biomolecules synthesis such as phenolic, flavonoids, proteins, photosynthetic pigments, salicylic acid and also an increase of catalase and trypsin inhibition activities. In susceptible accessions, the study revealed a decrease in the photosynthetic pigments and an increase in MDA content comparatively to resistant accessions. The ACP performed on the basis of the evaluated biochemical parameters showed that the axis F1 allows the separation of the resistant and the susceptible accessions, and indicated that the resistance of accessions result in the activation of biomolecules synthesis such as phenolic, flavonoids, proteins, photosynthetic pigments, salicylic acid and stimulation of catalase and trypsin inhibition activities. The F2 axis is associated with sugars content and, chymotrypsin inhibition and SOD activities. In susceptible accessions, an increase in MDA content and SOD activity were observed. This study represents an important step in combating leaf fungal diseases by opting to the green technology and by breeding for genotypes with inducing natural defense compounds.

Keywords: Jatropha; Resistance; Lasiodiplodia *theobramae*; Biochemical parameters.

1. Introduction

Since the industrial revolution in the eighteenth century, both of world population and energy consumption have steadily increased. The consequences were a decrease in the availability of fossil fuels and an increase in the price of oil. With the continuing rise in global energy consumption, the energy crisis is having a deeper impact on global social and economic development [1]. In order to set up sustainable development programs and support research on renewable energies, liquid bioenergy production from vegetable oil is an ecological alternative that ensures the development [2]. Due to the need for energy alternatives, *Jatropha curcas* has attracted the attention of many researchers and investors and has become a popular culture in the world [3, 4].

J. curcas genotypes are known to have toxins like phorbol esters, curcins and trypsin inhibitor, which confer to the specie a resistance to many pests and various pathogens [5-7]. However, monoculture and the increase in cultivated areas have given rise to a number of phytosanitary problems in these recent decades [1], among which the most important are leaf diseases [5, 7]. In Burkina Faso, the major leaf diseases affecting *J. curcas* plantations are necrosis and leaf blight. Symptoms occur in *Jatropha* plants of all ages and are characterized by discolorations followed by necrosis or blighting from one point to spreading all the leaves. Over time, the disease can progress and cause death of the plants [7]. Many fungal pathogens have been identified as responsible of leaf blight and necrosis of *J. curcas*. Among them, *Fusarium solani*, *Fusarium oxysporum*, *Curvularia lunata*, *Botrytis cinerae* and

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Lasiodiplodia theobromae (*Botyodiplodia theobromae*) are the best known. *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl, a member of *Botryosphaeriaceae* family, has been recognized in Burkina Faso by many authors as an important pathogen with high aggressive potential on *J. curcas* [8].

Generally, the control of these diseases is dependent on the use of chemical pesticides, although concerns have been raised due to increasing costs, deterioration of soil quality and environmental risks, and also due to consumer preference of fruit free of residue [9, 10]. Current scenarios to combat leaf fungal diseases are opting to the green technology by breeding for genotypes with induced natural defense compounds, thereby lowering both production costs and pesticide use [9, 11, 12]. Plants are known to respond to herbivory and microbial attacks through a range of factors, including those being of morphological, biochemical and molecular nature [9, 10]. Among biochemical factors, many previous studies in many species have confirmed involving of amino acids, total sugars, tannins, total phenols, protease inhibitors, and some osmotic and oxidant enzymes in resistance to fungal diseases [13, 14]. Thus, knowledge of resistance mechanisms may be important in selection and breeding for resistant genotypes [15]. Therefore, the aim of the present study was to evaluate some biochemical parameters of leaves in order to understand their possible associations to susceptibility or resistance of *J. curcas* to *Lasiodiplodia theobromae*, a leaf blight and necrosis agent.

2. Materiel and Methods

2.1. Plant Materials

The plant material is constituted of the seeds of eight (8) accessions of *J. curcas* from Burkina Faso including four (4) resistant and four (4) susceptible accessions. The seeds were used to set up a greenhouse nursery. The seeds were sown to a depth of 2 cm using pots with a 2-liters capacity. Each pot contains a mixture of sand, potting soil and organic manure in the proportions 3/1/1. This mixture, previously sterilized at 120 ° C for four (04) hours, allows good aeration of the roots and contains enough nutrients for the development of the plant. Pots were maintained in the greenhouse and were then watered every day.

2.2. Pathogenicity Test

Four weeks after sowing, five plants of each accession were inoculated with an isolate of *L. threobromae* previously produced. *L. threobromae* cultures were realized on Potato Dextrose Agar (PDA) medium during two weeks to produce inoculums. 20 ml of sterile water were poured into each Petri dish (containing a culture of pure strain) to obtain the maximum conidia, and the surface of the colony is minutely brushed using a fine brush. The obtained spore suspension was filtered with muslin to separate the conidia from the mycelia fragments. The conidial suspension collected is added with two drops of 10% Tween 80. Counts of conidia are then done under a Malassez counting cell microscope, and the concentration is adjusted to 2.10^6 conidia.ml^{-1}. The pathogenicity test was performed in the greenhouse according to the method described by Hernández-Cubero, *et al.* [6] after rubbing on the leaves of the carborandum, an abrasive powder which creates micro wounds on the leaves.

2.3. Estimation of Resistance Parameters

The leaves were collected the first day before inoculation and the 7th days after inoculation for each accession to evaluate the resistance parameters.

2.3.1. Determination of Photosynthetic pigments

Photosynthetic pigments (Chlorophyll a and b) contents were determined in fresh leaves samples. 300 mg of fresh leaves were homogenized in 1.5 ml of 95% ethanol. The mixture is stirred and kept for 10 minutes in ice. After centrifugation for 1 minute at 20.000 g, the absorbance of 300 µl of the supernatant was measured at 665 nm for chlorophyll a and 649 nm for chlorophyll b according to method described by Mimouni, *et al.* [16]. The results are expressed as µg / 100 mg of fresh leaves extracts (µg / 100mg).

2.3.2. Total Phenolic Content

The total phenolic content of fresh leaves extracts was determined at 760 nm using gallic acid as reference compound, as described by Yasmina, *et al.* [17]. The total phenolics were expressed as mg of gallic acid equivalent per 100 mg of fresh leaves weight (mg GAE/100 mg of fresh leaves).

2.3.3. Flavonoid Content

The total flavonoid content of the cowpea seed extracts was determined at 415 nm using the $AlCl_3$ method described by Hilou, *et al.* [18]. The total flavonoid content was determined on a quercetin calibration curve and expressed as mg of quercetin equivalents (QE) per 100 mg of fresh leaves weight (mg QE/100 mg of fresh leaves).

2.3.4. Soluble Sugar Content

500 mg of leaves were homogenized in 5 ml of 80% hot ethanol. After cooling, the homogenate was centrifuged at 4000 rpm for 10 min. The supernatant was used to estimate the soluble sugar content of fresh leaves. The soluble sugar content was determined using the phenol-sulfuric acid method as described by DuBois, *et al.* [19] and the absorbance was red at 490 nm. The total sugar content was expressed as µg glucose equivalent/100 gram of fresh leaves (µg GE/ 100 g of fresh leaves).

2.3.5. Salicylic Acid Content

500 mg of fresh leaves were homogenized in 5 ml of distilled water and then centrifuged at 10 000 g for 10 minutes. The salicylic acid content was determined in the supernatant according the method described by Yang, *et al.* [20] and expressed as mg/100 mg of fresh leaves (mg/100 mg of fresh leaves).

2.4. Protein content and protease inhibition potential

2.4.1. Extraction of Protein

Proteins were extracted according to the protocol described by Klomklao, *et al.* [21]. 500 mg of leaves were homogenized in 5 ml of 0.1 M NaCl for 5 h at 150 rpm / min at room temperature. The samples were centrifuged at 10000 g during 30 min and the supernatant were collected to determine the protein content and the protease inhibition potential of leaves.

2.4.2. Protein Content

Protein concentration was measured by Bradford method as described by Mimouni, *et al.* [16] using bovine serum albumin as a standard.

2.4.3. Trypsin and Chymotrypsin Inhibition Assay

Trypsin and chymotrypsin assay were determinate according a standard method described by Klomklao, *et al.* [21]. To measure trypsin inhibition (TI), 100 µl of trypsin (from bovine pancreas, Sigma) at 0.0125 $mg.ml^{-1}$ and 100 µl of total protein extract were combined and were incubated for 5 min prior to the addition of 50 µl of *N*-α-Benzoyl-DL-Arginine *p*-Nitroanilide (BAPNA) at 0.8 $mg.ml^{-1}$. The liberated *p*-nitroanilide was monitored for 25 min at 410 nm using a spectrometer.

To assay chymotrypsin inhibition potential (CI), 100 µl of α-Chymotrypsin (from bovine pancreas, Sigma) at 0.01875 $mg.ml^{-1}$ and 100 µl of total protein extract were combined and were incubated for 5 min prior to the addition of 50 µl of *N*-Glutaryl-L-Phenylalanine *p*-Nitroanilide (GPNA) at 3.2 $mg.ml^{-1}$. The liberated *p*-nitroanilide was monitored for 25 min at 410 nm using a spectrometer. Each reading was made against a control and trypsin and chymotrypsin inhibition activities of samples were estimated as percentages of inhibition according to the formula:

Percent inhibition = $\frac{Vmax\ contr - Vmax\ sample}{Vmax\ contr} \times 100$

2.5. Determination of antioxidant enzymes activities

2.5.1. Extraction of Antioxidant Enzymes

500 mg of fresh leaves were milled using an extraction buffer containing 50 mM sodium phosphate (pH 7.8). The superoxide dismutase and catalase activities were assayed on the supernatant obtained after centrifugation at 4000 rpm for 10 min at 4°C.

2.5.2. Measurement of Superoxide Dismutase (SOD) Activity

SOD was assayed using the standard method revealed by Ranjitha and Vijiyalakshmi [22]. This method is based on the inhibition of Epinephrine-Adrenochrome transition by the enzyme. The enzyme activity in the fresh leaves was determined using a spectrophotometer at 420 nm. The enzyme activity was expressed in terms of unit min^{-1} mg^{-1} protein.

2.5.3. Measurement of Catalase (CAT) Activity

CAT was assayed using standard protocol described by Ranjitha and Vijiyalakshmi [22]. The breakdown of H_2O_2 on addition of the enzyme is followed by absorbing the decrease in light absorption of peroxide solution in the UV region. The activity was measured as change in optical activity/density at 240 nm for 30sec interval during 3minutes. The CAT activity was expressed in terms of µmole of H_2O_2 consumed/minute/mg protein.

2.6. Lipid Peroxidation Assay

The lipid peroxidation was estimated by the evaluation of Malondialdehyde (MDA) content according to the method described by Zineb, *et al.* [23]. The lipid peroxidation was expressed as concentration of MDA in fresh leaves ($\mu mol.mg^{-1}$ of fresh leaves.

2.7. Statistical Analysis

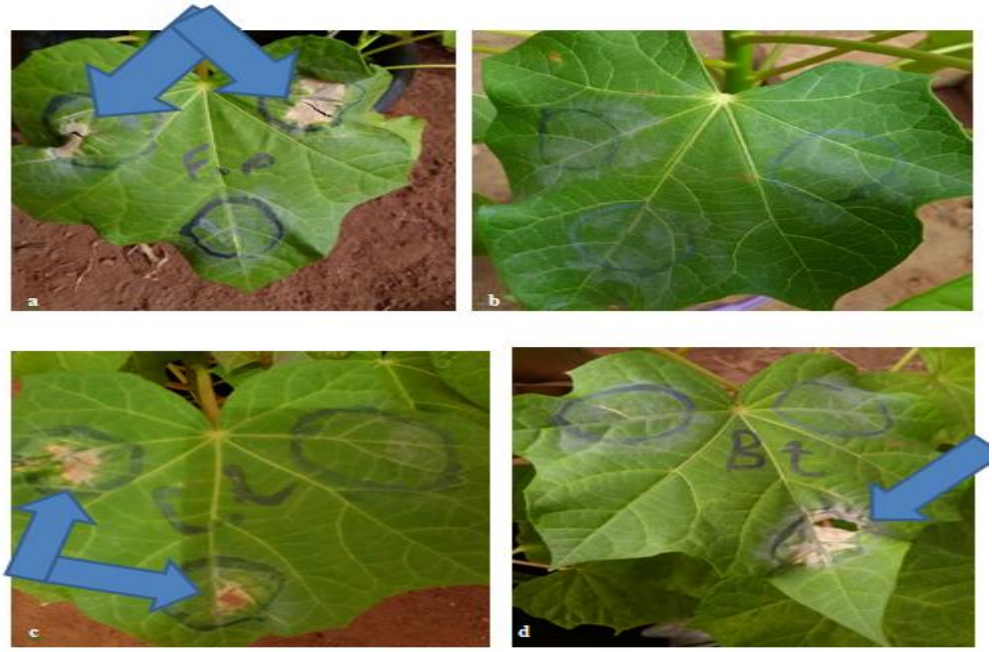
The results are presented as mean ± SD for triplicate analysis and were subjected to one-way analysis of ANOVA variation with Tukey's Significant Difference test and $P < 0.05$ was considered significant. The Pearson correlation test was used to study correlations between parameters and the effect of parameters variation on the susceptibility or the resistance of accessions. The statistical analysis was performed using XLSTAT Version Pro-2017 and the graphs were drawn using Graph Pad Prism software version 5.0.

3. Results

3.1. Pathogenicity Test

The results of the pathogenicity test are presented in Fig 1.

Fig-1. Leaf structures of some accessions of J. curcas at the 7th day after inoculation (the blue arrows indicate necrosis on inoculation zone)



a: leaf of JR2 (resistant accession) at 7th day; **b:** leaf of JS1(susceptible accession) at 7th day; **c**: leaf of JS3 at (susceptible accession)7th day; **d**: leaf of JS4 (susceptible accession) at 7th day

Inoculated plants showed different reactions to the fungal pathogen according to their susceptibility or their resistance/tolerance. Susceptible accessions presented necrosis on inoculation zones (Photo 1a) and resistant accessions did not show any symptoms of leaf diseases (Photo 1b, 1c, 1d) at the 7th day after inoculation.

3.2. Chemical Compounds Contents

The variation in the levels of various chemical compounds between the 1st day before and the 7th day after inoculation of different accessions of *J. curcas* are presented in Table 1.

Table-1. Variation of various chemical compounds contents of the 8 accessions

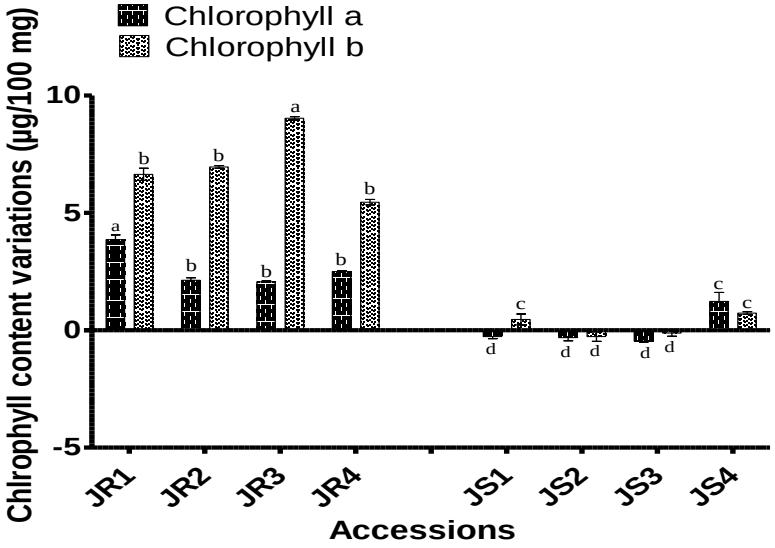
Accessions	Phenolic (mg EAG/100mg)	Flavonoids (mg EQ/100mg)	Proteins (10^{-2} mg/100mg)	Sugars (µg EG/100mg)	Salicylic acid (mg/100mg)
JR1	8,098 ^{a}	8,651 ^{a}	11,228 ^{a}	64,679 ^{a}	13,667 ^{a}
JR2	8,166 ^{a}	8,151 ^{a}	11,489 ^{a}	33,616 ^{cd}	13,087 ^{a}
JR3	8,037 ^{a}	8,420 ^{a}	10,947 ^{a}	34,931 ^{bcd}	13,638 ^{a}
JR4	8,764 ^{a}	7,408 ^{a}	13,798 ^{a}	62,585 ^{ab}	13,667 ^{a}
JS1	1,207 ^{b}	0,564 ^{b}	1,082 ^{b}	27,218 ^{d}	5,333 ^{b}
JS2	1,746 ^{b}	0,974 ^{b}	-3,125 ^{b}	16,258 ^{d}	5,058 ^{b}
JS3	1,071 ^{b}	1,384 ^{b}	1,426 ^{b}	62,017 ^{abc}	4,826 ^{c}
JS4	1,339 ^{b}	1,625 ^{b}	0,568 ^{b}	43,020 ^{abcd}	5,348 ^{b}

The contents of total phenolic, total flavonoid, protein and salicylic acid of the 8 accessions showed significant variation according to their resistance/susceptibility between 1st day before and 7th day after inoculation. The resistant accessions possessed the significant high content in these compounds comparatively to the susceptible accessions of *J. curcas.* The sugar content did not show any significant variation among the accessions according to their resistance/susceptibility. In fact, the resistant accessions JR1 and JR4 showed the high content of sugar but not significantly different with those of some susceptible accessions like JS3 and JS4.

3.3. Photosynthetic Pigment Content

The variation in the levels of photosynthetic pigments (chlorophyll a and b) at 1st day before and 7th day after inoculation are presented in the fig 2.

Figure-2. Photosynthetic pigment **(**Chlorophyll a and b) content of accessions

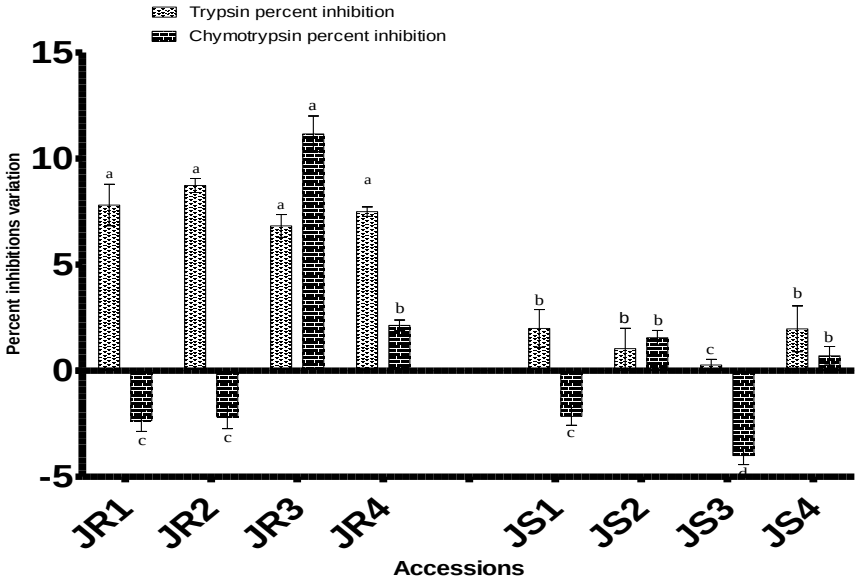


There is significant difference in the variation of photosynthetic pigments content according to the resistance or susceptibility of the accessions. Resistant accessions recorded significant increases in the two pigments between the 1st day before and the 7th day after inoculation, unlike the susceptible accessions recorded slight decreases in the two pigments content. The increases in the contents of the photosynthetic pigments are positively correlated with the resistance of the accessions.

3.4. Proteases Inhibition Activities

The **v**ariations in the percent inhibition of trypsin and chymotrypsin of the 8 accessions are shown in the fig 3.

Figure-3. Inhibition potential of *Jatropha curcas* accessions on trypsin and chymotrypsin

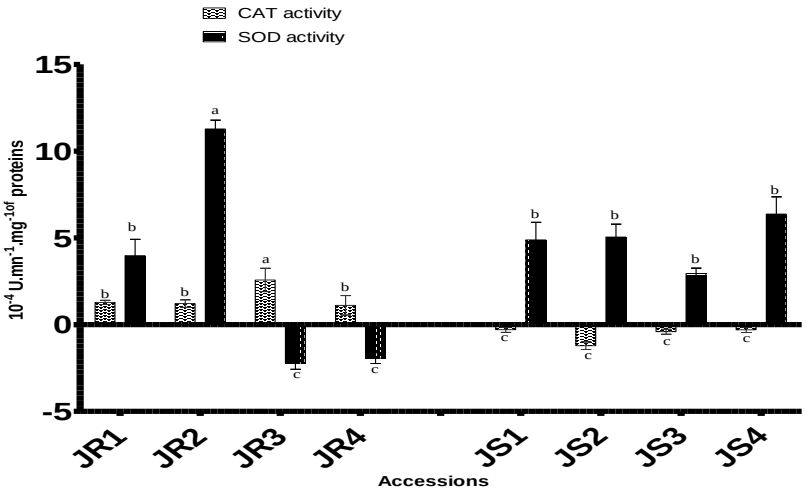


These results showed significant variation on the two proteases inhibition potential of the different accessions. The resistant accessions recorded a significant increase on the trypsin inhibition compared to the susceptible accessions which presented a decrease of trypsin inhibition activity in some accessions between 1st day before and 7th day after inoculation. The increase of trypsin inhibition percentage of the resistant accessions could be explained by the strong presence of trypsin inhibitors in resistant accessions comparatively to susceptible accessions of *Jatropha curcas*. The resistant accession JR3 showed the high increase of chymotrypsin inhibition activity among the 8 accessions. The resistant accession JR4 and the susceptible accessions JS2 and JS4 also showed increase in their chymotrypsin inhibition activity. In contrary, the resistant accessions JR1 and JR2, and the susceptible accessions JS1 and JS3 showed diminution in the chymotrypsin enzyme inhibition activity. The chymotrypsin inhibition potential of *Jatropha curcas* is not related to the resistance or the sensitivity of the accessions.

3.5. Activities of Antioxidant Enzymes

The antioxidant enzymes (catalase and SOD) activities variations of different accessions between 1st day before and 7th day after inoculation are presented in the fig 4.

Figure-4. Catalase and superoxide dismutase activities

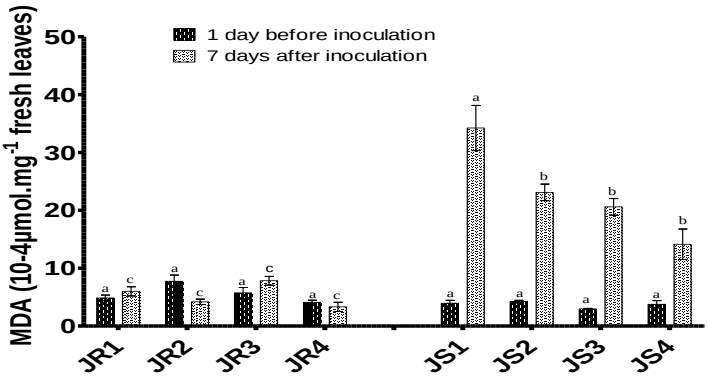


The results showed that the variation of the catalase enzyme activity is depending on the resistance or susceptibility of the accessions. The resistant accessions showed an increase in the activity of catalase while the activity of this enzyme decrease in the susceptible accessions between the first day before and the seventh day after inoculation. The JR2 resistant accession exhibited the high significant increase of superoxide dismutase activity among the accessions. The JR3 and JR4 resistant accessions showed a decrease of SOD activity between the 1st day before and the 7th day after inoculation. The significant increase of CAT enzyme activity after fungal infestation could explain their resistance mechanism.

3.6. Lipid Peroxidation of Leaves

The fig 5 shows the concentration of MDA expressed as µmol.mg^{-1} of fresh leaves of the different accessions at 1 day before inoculation and 7 days after inoculation.

Figure-5. MDA content of accessions at 1st day and 7th day after inoculation



The results showed that at the 1st day before inoculation, there is no significant difference between the MDA concentration in the leaves of both resistant and susceptible accessions. However, at 7th day after inoculation, the susceptible accessions showed significant increases in MDA concentration than resistant accessions whose MDA concentration did not vary significantly. In addition, the susceptibility of accessions was significantly correlated with the increased of leaves MDA concentration.

3.7. Correlations Between the Evaluated Parameters

The table 2 shows the correlations between the different parameters evaluated.

Table-2. Pearson correlation coefficients between different chemical compounds

Variables	Phenolic	Flavonoids	Proteins	Sugars	Salicylic acid	ChA	ChB	Trypsin	Chymotrypsin	Catalase	MDA	SOD
Phenolic	**1**											
Flavonoids	**0.983**	**1**										
Proteins	**0.963**	**0.953**	**1**									
Sugars	0.335	0.369	0.489	**1**								
Salicylic acid	**0.992**	**0.993**	**0.965**	0.338	**1**							
ChA	**0.874**	**0.901**	**0.856**	0.492	**0.868**	**1**						
ChB	**0.947**	**0.972**	**0.916**	0.226	**0.967**	**0.834**	**1**					
Trypsin	**0.974**	**0.966**	**0.949**	0.276	**0.974**	**0.892**	**0.935**	**1**				
Chymotrypsin	0.362	0,355	0.268	-0.266	0.341	0.207	**0.502**	0.260	**1**			
Catalase	**0.081**	0.033	-0.072	0.120	0.027	0.230	-0.102	0.014	-0.128	**1**		
MDA	**-0.884**	**-0.903**	**-0.848**	-0.411	**-0.866**	**-0.856**	**-0.835**	**-0.866**	-0.298	-0.003	**1**	
SOD	-0.262	-0.220	-0.294	-0.351	-0.233	-0,148	-0.258	-0.082	**-0.620**	-0.138	0.092	**1**

Values in bold indicate a significant value ($p < 0.5$)

Significant and positive relationship were found between variation in phenolic content and variation in flavonoids (r = 0.983), total protein (r = 0.963), salicylic acid (r = 0.992), chlorophyll a (r = -0.856), chlorophyll b (r = 0.947) and trypsin inhibition (r = 0.974). High and significant correlations were also found with variation of flavonoids contents and protein (r = 0.953), salicylic acid (r = 0.993), chlorophyll a (r = 0.901), chlorophyll b (r = 0.972) and trypsin inhibition (r = 0.966). In addition, the variation in MDA content was negatively correlated with those of phenolic (r = - 0.884), flavonoids (r = - 0.903), total proteins (r = -0.848), salicylic acid (r = - 0.866), and variation in trypsin inhibition (r = -0.866). The variation of chymotrypsin inhibition was positively correlated with that of chlorophyll b (r = 0.502) and negatively correlated with the variation of SOD activity (r = -0.620). The variation in total sugar content and catalase activity was not significantly correlated with any of the parameters evaluated.

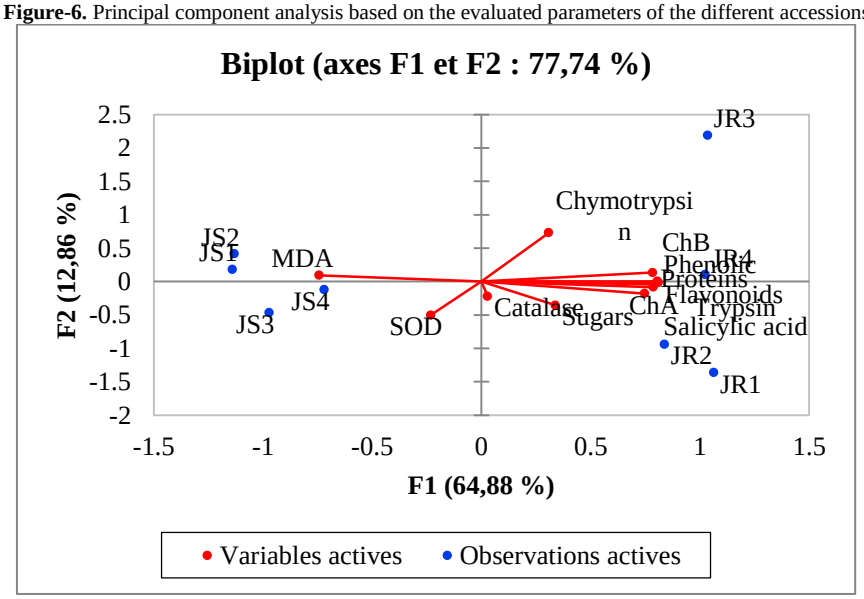
3.8. Comparative Analysis of the Variation of Resistant and Susceptible Accessions

The principal component analysis was performed on the basis of variations in the different parameters evaluated between the 1st day before inoculation and the 7th day after inoculation. The table 3 shows coordinates of accessions. The results showed positive values for resistant accessions and negative values for susceptible accessions for F1 axis. This axis allows a separation of the resistant accessions of the susceptible accessions.

Table-3. Coordinates of the different accessions

	JR1	JR2	JR3	JR4	JS1	JS2	JS3	JS4
F1	2,967	2,339	2,890	2,860	-3,183	-3,156	-2,712	-2,006
F2	-1,694	-1,170	2,722	0,130	0,226	0,519	-0,581	-0,152

Figure-6. Principal component analysis based on the evaluated parameters of the different accessions



The fig 6 presents the repartition of the 8 accessions in the biplot (axis F1 and F2: 77.74 %). The contribution of the different variables for the F1 and F2 axes shows a strong contribution of the variables phenolic (12.500), total flavonoids (12.656), proteins (12.084), salicylic acid (12.478), chlorophyll a (10.776), chlorophyll b (11.850), MDA (10.636) and inhibition of trypsin activity (11.932) for F1 axis and sugars (12.212), chymotrypsin (51.795) and SOD (24.576) for the F2 axis (12.86 %°).

4. Discussion

The study was initiated in order to identify the biochemical mechanisms involved in the resistance of *J. curcas* to *L. threobromae*, a fungal pathogen of this species. The results of the pathogenicity test showed necrosis or leaf blight on leaves of susceptible accessions while the leaves of resistant accessions did not show symptoms of leaf diseases. Similar results have been reported by Lanubile, *et al.* [24]. Lehmann, *et al.* [13] whom also recorded a variability of responses between susceptible and resistant accessions of maize inoculated with *Fusarium proliferatum*, *Fusarium subglutinans*, and *Aspergillus flavus*.

The resistant accessions showed a significant increase in the contents of polyphenols, flavonoids, proteins, salicylic acid and in photosynthetic pigments between the 1st day before and the 7th days after inoculation. The susceptible accessions did not show significant variations in the content of these compounds. The resistance of *J. curcas* to *L. threobromae* could be explain by the synthesis of these compounds in the resistant accessions. Similar results were reported in others studies. Indeed, Borković, *et al.* [25] reported that resistance to *Monilinia fructicola* in *Prunus persica* L. is associated with increased levels of polyphenols, flavonoids and various biomolecules. Vagiri, *et al.* [10] have also reported that phenolic compounds such as catechin and epicatechin are responsible for resistance to gray mold (*Botrytis cinerea*) in strawberries. According to Gharbi, *et al.* [26], Zraibi, *et al.* [27], the slight

decrease in the photosynthetic pigments content in susceptible accessions could be explained by a degradation or a perturbation of the photosynthetic system. Also, Cameron, *et al.* [28]; Fu, *et al.* [29] have reported that resistance of plants is associated with increased accumulation of salicylic acid. Indeed, Klessig, *et al.* [30] reported that the transfer to 30 ° C of tobacco resistant cultivars to the mosaic virus block the accumulation of salicylic acid and lose the resistance character, the plant being unable to ensure the biosynthesis of the molecules of defense. Our results are also similar to those reported by Machado, *et al.* [31]; Zhu [32]. They reported that plant resistance to stress was associated with increased in protein levels. Machado, *et al.* [31], have reported that resistance to stress is associated with accumulation of certain proteins including proline. However, our results are contrary to those of Zhu [32]; Srinivasa, *et al.* [33] whom reported an increase in sugar levels in resistant accessions.

The variations in the levels of the biomolecules was accompanied by a significant increase in the inhibition activity of trypsin and in the activity of CAT in resistant accessions. Previous studies have reported similar results in other plant-pathogen ecosystems. Hegazi, *et al.* [15] reported plant resistance mechanisms involving antioxidant system enzymes including catalase (CAT) and proteases inhibition including trypsin inhibition. Lehmann, *et al.* [13] also reported that CAT activity were augmented after inoculation of fungi, especially in the resistant line of maize.

In susceptible accessions, an increase in MDA content was observed. Similar results were reported by Benhamou and Rey [34]; Zraibi, *et al.* [27]. They reported an increase in MDA content, an indicator of oxidative degradation of membrane lipids, in leaves of susceptible accessions. Srinivasa, *et al.* [33]; Benhamou and Rey [34] explained this result by perturbation of photosynthetic activity. Indeed, Gharbi [35] have reported that lipid degradation is due firstly to a disruption of thycoidal membranes, a loss of integrity of chloroplasts and hence a decrease in photosynthetic activity and secondly to a reduction of the activity of different enzymes of the antioxidant system of leaves. This would cause an accumulation of Reactive Oxygen Species or (ROS); such as superoxide ion O_2^-, hydrogen peroxide H_2O_2, $OH^·$ Hydroxyl radical in the tissues and thus fortify the oxidation of membrane lipids. Thus, the reduction would promote the negative effects of lipid peroxidation reactions.

The ACP results indicate the involving in the resistance of accessions a multi-component defense system, in which regulation of chemical compound levels, trypsin inhibition photosynthetic pigments and catalase activity would play a central role in limiting the invasion and growth of pathogens. Similar results have been reported in various plant species. Indeed, according to [14, 34] the global expression of resistance to microbial pathogens is the result of a synergy of coordinated action in time and space between all whether direct or indirect defense mechanisms. Among the indirect mechanisms, there is parietal enhancement by incrustation of polysaccharide molecules, such as callose, phenylpropanoid pathway metabolites, such as lignin and phenolic compounds, and structural proteins and glycoproteins, such as hydroxyproline-rich glycoproteins (HRGPs). This parietal enhancement most often results in the formation of new barriers called papillae or parietal appositions which strongly contribute to delay the progression of the pathogen in the plant tissues and to prevent the diffusion of deleterious substances such as walls degrading enzymes, or toxins. Direct mechanisms include, among others, the synthesis of new proteins such as stress proteins, enzymes (antioxidant enzymes for example) and protease inhibitors [15, 34] as well as the production of phytoalexins, secondary metabolites with antimicrobial potential [14].

The positive and significant correlations between some of resistance parameters are very interesting results for breeding programs. Indeed, according to Freitas, *et al.* [36] knowledge of the magnitude of the correlation between characters is important in the choice of improvement methods and the formulation of strategies for the simultaneous selection for several desired characters. This research is an interesting study to determine sources influencing resistance to pathogens in *Jatropha* genotypes for consequent breeding purposes.

5. Conclusion

This study revealed that the response of *J curcas* to *L. threobromae* infection is depending to the resistance or the susceptibility of accessions. It also revealed that the resistance of resistant accessions is the result of an activation of biomolecules synthesis such as phenolic, flavonoids, proteins, photosynthetic pigments, salicylic acid and increase of catalase activity and trypsin inhibition activity which would aim at either curbing the penetration of the pathogen or destroying it.

In susceptible accessions, pathogens would attack by degrading or disrupting photosynthetic activity and increasing peroxidation of membrane lipids. These results open voices for improvement *J. curcas* resistance in breeding programs and in the identification of *J. curcas* natural defense stimulators, which could lead to effective management of its phytosanitary problems by non-recourse chemical pesticides.

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