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STUDY OF THE FUNGAL FLORA AND EFFECT OF FUNGAL PATHOGENS ON GRAIN QUALITY OF SOME VARIETIES OF RICE (*Oryza sativa L.)* PADDY CROP IN BENIN

GNANCADJA A. S. LEOPOLD[1], GISÈLE Y. HODE[2], ELEGBEDE A. FÉLIX[3], FATON M OSCAR EULOGE[4] AHANHANZO CORNEILLE[5] EDORH A. PATRICK[6] & AKOEGNINOU AKPOVI[7]

[1,2,4]University of Abomey, Faculty of Science and Technology, Department of Plant Biology, Plant Physiology Laboratory Benin

[3]University of Abomey-Calavi, Analysis Laboratory of Hydrocarbons, Head Office of Hydrocarbons and Others Fossilles, Benin

[5]University of Abomey, Faculty of Science and Technology, Plant Biotechnology Laboratory Benin

[6]University of Abomey-Calavi, Laboratory of Biochemistry and Cell Biology, Faculty of Technical Sciences, Benin

[7]University of Abomey-Calavi, Plant Biology Department, Botany and Plant Ecology Laboratory, Faculty of Technical Sciences, Benin

ABSTRACT

Rice *(Oryza sativa L.)* is major cereal crop year and is consumed in various forms. DESPITE extensive use ict, ict output is affected by the disease with a yield reduction caused by the attack of fungal and bacterial pathogens. The present study was conducted with the main aim have to make a survey on some diseases affecting rice seeds in a producing area of rice in Benin and the secondary aim for assessment as to the pathogenicity of some fungi on some varieties grown rice in Benin. Disease diagnosis consisted of the isolation and identification of pathogens. Were produced from inoculated isolates tests tarnish grains under controlled conditions. The isolation from the file Managed samples and diagnostic tests Was Performed APPROBATION seven kinds of rice diseases caused by The Following pathogens: *Curvilaria lunata, Helmintosporium oryzae oryzae Nigrospora, verticiloides Fusarium, Alternaria oryzae* bacteria and nematodes. Among thesis pathogens, *Curvilaria lunata, Helminithosporium oryzae,* are to be Revealed The Most aggressive disease.

KEYWORDS: Rice Seed, Disease, Pathogenicity, Alteration

INTRODUCTION

Rice *(Oryza sativa L.)* is an annual plant cultivated for its edible seeds. It is the third most cultivated cereal in the world, with a production of 15% (FAO, 2001). Asia is the continent which provides the largest world production (90%), followed by South America (3.2%) and Africa (2.8%) (Hirsch, 1999). Asian production is estimated at 657 million tonnes in 2012 (FAO, 2012) and the largest producers are China, India, Indonesia, Bangladesh and Vietnam. In Africa, Egypt is the largest producer followed by Nigeria and Madagascar (Adegbola and Sodjinou 2003). In West Africa, Nigeria is leading followed by the Ivory Coast and Guinea with 48%, 17% and 10% of the total regional output (Adegbola and Sodjinou 1985). However African production does not meet demand. The African continent has become a major rice importers. Rice consumption contributes over 20% to the global supply of calories (WARDA, 2006). It serves as a staple food for over half of the world population. It is also the food source that is growing faster in Africa where it is grown in around 40 countries (WARDA, 2005). It represents an important culture of food safety point of view (CARD, 2008). In Benin, rice cereal is currently the third in terms of production, after maize and sorghum. Just for the supported sites, production reached 1,376 tons in 2004. Rice production increased from year to year especially with the wholesale marketing of paddy. New thinking led to the redefinition of need (Nonfon, 2005). In 2009, paddy production is estimated at 109,371 tons against 16,545 tons in 1995 and 72,960 tons in 2007. The demand for rice in 2015 is estimated at 132 750 tonnes (Adegbola et al., 2003). Such growth is related to the intensification of sown areas, the commitment of producers and the support of development partners (CBRC, 2010). In addition, there is an organization of producers, a public-private partnership in development, a clear political will and the involvement of technical and financial partners in the distribution chain promotion. (NRDS, 2011) Despite all this, rice production is still very low to cover the internal needs of the population. Also, Benin has a significant potential for the promotion of rice in terms of irrigable land: 322,000 ha of which only 10% are currently exploited (CBRC, 2004). The area planted between 2008-2009 amounted to 33,294 ha with a total production estimated at 109 371 tonnes of paddy rice. Available land is suitable for rice cultivation. Indeed, water is necessary and is relatively abundant (rain, shoals, streams and river), the climate is favorable. Furthermore, the limited availability in rural families, low yields, low prices for sale... influence its production. All these factors are reinforced by the commercialization and mass imports of rice of better quality (less breakage) and cheap. Or rice cultivation provides significant revenue to producers, hence the need to train on good agricultural practices for production quality and quantity (CBRC, 2012). Indeed, the quality of the seed is an essential element to any successful rice production and the essential production equipment of the farmer. Studies have shown the presence of many pathologies observed on seed paddy, causing major problems such as blast, brown spot, the curvilariose, *Xanthomonas oryzae* (bacterial), parasitic nematodes. It is therefore important to assess cases of contamination from harmful microorganisms (fungal and bacterial) on paddy rice varieties cultivated in Benin. This is the main objective of this work entitled 'Determination of the impact of pathogens on grain quality of some varieties of paddy rice *(Oryzae sativa L.)* grown in Benin "Specifically, it will not only to assess the diversity of common pathogens on rice seeds but still produce the inoculum containing the isolated fungal microorganisms seed to evaluate the impact of certain fungal pathogens on healthy rice seed, to evaluate alteration percentages obtained by each type of fungal pathogen on these seeds.

MATERIAL AND METHODS

Equipment

The plant material consists of rice panicles samples having characteristic symptoms of diseases of seeds and rice leaves. This is 16 paddy varieties NERICA L20, CMR 86-030- A TOG 16799, Kountounkaassa, OG 20, Mororiz, GC17-A, YG 28 NERICA L14, BL 19, BJ11, IR 64, IR 841, Long TOS, TOS and Beri Court 2.

METHOD

Sampling

The sample collection procedure was to cross the rice fields of the sites and collect samples of rice stalks paniculaires based on observation of symptoms characteristic of the disease on rice seed (figure: 13) as defined by the other researchers. Each sample is consisting of 2 to 4 panicles placed in labeled envelopes. A total of sixty-five (65) samples were collected during the prospecting in the areas visited. After screening, 42 were selected. The distribution of retained samples is given in the following table.

Table 6: Distribution of Samples Following Collection Sites

Rice Panicles Samples	variety	Collecting zone
27	Nerica L 20	Common Zè
1	CMR 86. A 030-	AfricaRice
1	TOG 16799	AfricaRice
1	Kountounkaassa	AfricaRice
1	OG 20	AfricaRice
1	Mororiz	AfricaRice
1	GC17-A	AfricaRice
1	YG 28	AfricaRice
1	NERICA L14	AfricaRice
1	BL 19	AfricaRice
1	BJ11	AfricaRice
1	IR 64	AfricaRice
1	IR 841	AfricaRice
1	TOS Long	AfricaRice
1	TOS Court	AfricaRice
1	beri 21	AfricaRice

Figure 13: Health Status of Rice Seed Panicles Taken To the Field to Zè

Evaluation Frequent Cases of Contamination of Rice Seed

The completion of the evaluation of cases of contamination was done in three **stages.**

Culturing

The seeds were disinfected sample for 30 seconds using a sodium hypochlorite solution (NaHCL $_{2)}$ 2% and then rinsed with sterilized distilled water for 30 seconds. Rinsing was repeated three times to remove the maximum amount of bleach. Seeds were plated on sterile paper for drying for 30 seconds. Then from 10 to 15 seed grains were deposited on the culture media (PSA Gzapeck, rice flour) previously cast in boxes kneaded. Then these seeds were filed steeped in boxes containing only paper moistened with distilled water (the method of the modified filter paper). Steeped in the boxes were sealed with parafilm and incubated at 28 ^{o} C for three days in cases of culture media and eight days for cases left on the soaked paper. The tests were repeated 2 times.

Microscopic Observation

Two methods have favored the review of spores and other structures. For the first method the seeds left on paper soaked in water were introduced into 2 ml of sterile distilled water. The mixture was vortexed and then a drop of the resulting mixture was placed on a microscope slide for observation. The second method is to prick with a scapelle blade, a thin portion of the mycelium which has grown around the seed and then place it on the microscope slide onto which has been previously deposited a drop of sterilized water. This method relates to the seeds left on the PSA circles Gzapeck and rice flour. After observing microscopic spores, follow the isolation of these spores on culture media. It is only the spores and bacteria colonies developed on SAP environments, Gzapeck rice flour and rice flour which were used for this part.

ISOLATION AND MONOCULTURE

Purification of the Fungal Spores

Under a laminar flow hood, a single (specific) spore observed under a microscope or bacteria colony visible to the naked eye on the culture media was deposited on the Gzapeck nutrient medium. The whole was incubated at 28 ° C for five to fifteen days.

Test on Bacteria Gram KOH 3%

A l`aide d`une platinum loop sterilized Bunsen burner flame, the bacteria were removed from d`une pure culture 48 hours old on the PSA medium and rubbed rapidly in a drop of a solution of (48) hours to seventy-two (72) hours of age and suspended in a drop of 3% aqueous KOH solution previously deposited on a glass slide. After a few seconds, it raises the toothpick of a few cm above the blade. If it forms a viscous net, the bacterium is gram negative. Gram-positive bacteria do not produce net. (Lelliot, **1987).**

Tarnish Testing Grain

Tarnish tests were performed with the monoculture of *Helminthosporium oryzae, Fusarium* and *verticiloides Curvilaria lunata*,. The plant material consists of grains of rice varieties NERICA L 20 *and IR-64* (widely grown in Benin). These grains are disinfected by soaking in a sodium hypochlorite 5% solution for two minutes followed by three rinses with sterile distilled water and then dried.

Fungal Material

Three fungal species isolated from rice plants were used. This is *Helminthosporium oryzae, Curvularia lunata* and *Fusarium verticiloides.* All these species were kept on filter paper discs to - 20 ° C freezer.

Preparation of Inoculum

For the production of the inoculum, *Helminithosporium oryzae and Fusarium moniliforme* were grown on a medium based on rice flour, *Curvularia lunata,* meanwhile was grown on Gzapeck medium and incubated at 28 ° C and.

After 15 days of incubation for about *verticiloides Fusarium and Curvularia lunata,* ten (20) days for about *Helminthosporium oryzae* spores of the charged surface is scraped aseptically using a metal spatula and distilled water. The suspension obtained is filtered through cheesecloth to remove mycelial spores. They were supplemented with

0.05% Tween 20 and 0.5 of gelatin necessary for successful inoculation and enable uniform deposition of water droplets on the surface of the seeds. The inoculum concentration is fixed than 10 ^{5} spores / ml with a Malassez slide.

Grain Processing

The varieties of rice grains NERICAs L 20 and IR 64 are separated into batches of 400 to 600 grains. Grain batches are soaked for 1:30 minutes (time required for the inoculum is impregnated grains) in the inocula obtained from various fungal species. Grain witnesses in water have not received spore suspensions but rather have been soaked in distilled water 0.5% gelatin and Tween 20 for 1 hour 30 minutes. All grain batches are then carefully dried and spread on the bench for 72 hours, enough time for the onset of symptoms of some parasites tested. Then lots are kept for 3 months at a temperature of 28 ° C for the final rating of symptoms.

Rating Scale Results

The rating of the alteration of the grains was carried out using an arbitrary scale, preset shown in Table 7 below.

Table7: Rating Scale

Note	Descriptions Damage
0	No apparent symptoms
1	A few isolated patches of brown on part of the grain
2	More or less large spots distributed over a portion of the grain
3	Round or elongated spots spread over 2/3 of the grain.
4	Alteration of all or substantially the entire grain surface.

The sum of the scores related to the number of treated and untreated grains, is the index alteration. A medium index is then calculated for each batch of grain.

Alteration index = <u>Σ obtained Notes</u>
Observed Number of grains

It should also be mentioned that the percentage of fading of having grains was noted

Statistical Analysis

Data processing tarnish indices was performed with Statistica software and focuses on the analysis of variance followed by the Student-Newman-Keuls (SNK) at the 5% threshold when the result of the analysis of the variance recorded at least one significant difference at the 5% threshold. Microsoft Excel 2007 was used for data entry.

RESULTS AND DISCUSSIONS

Results

Evaluation Frequent Cases of Contamination of Rice Seed

Sixteen (16) rice varieties were used in the study. These are: NERICA L20, CMR 86-030- A TOG 16799, Kountounkaassa, OG 20, Mororiz, GC17-A, YG 28 NERICA L14, BL 19, BJ11, IR 64, IR 841, TOS Long, TOS Court and Beri September 21. groups of pathogens are observed on different rice varieties. These are: *Curvilaria lunata*, *Helminthosporium oryzae*, *Nigrospora oryzae*, *Fusarium verticiloides*, *Alternaria oryzae*, bacteria and nematodes. All collected rice varieties are attacked by at least one of the identified pathogens (Table 8) *Curvilaria lunata* differs from lot with seven (7) varieties attacked *Alternaria oryzae..; Nigrospora oryzae oryzae and Sarocladium* have attacked the variety NERICA L 20.

Table 8: Distribution of Pathogens Following Rice Varieties Studied

Pathogens	Variety
Alternaria oryzae	Nerica L 20
Bacterium	Kountounkaassa, Mororiz, Nerica L 20, TOG 16799
Curvilaria lunata	Beri 21 GC17-A, IR 64, 20 OG, YG 28, Nerica L 20, L 14 Nerica
Fusarium verticiloides	BJ11, Nerica L 20, TOS Long
Helminthosporium oryzae	BL 19, IR 841, Nerica L 20
nematodes	CMR 86- 030- A, Nerica L 20, TOS Court
Nigrospora oryzae	Nerica L 20
Sarocladium oryzae	Nerica L 20

Figure 14 below shows the pathogens found on Nerica L 20 and frequency of attack. *Curvilaria lunata* This is the most pathogenic of the panicles of Nerica L 20 with a 23.08% attack rate.

It is followed by *Helminthosporium oryzae oryzae* and *Nigrospora* of *Sarocladium oryzae* with frequencies of attack of 15.38% each. *Alternaria oryzae* is the least common pathogen on the irrigated perimeter of Zè.

In the experimental fields AfricaRice *Curvilaria lunata* and bacteria are the pathogens present with 40% attack frequency and 20% respectively (Figure15). Then come*verticiloides Fusarium, Helminthosporium oryzae* and nematodes with attack frequency of 13.33% each. *Alternaria oryzae oryzae and Nigrospora Sarocladium oryzae* were not found in these experimental fields.

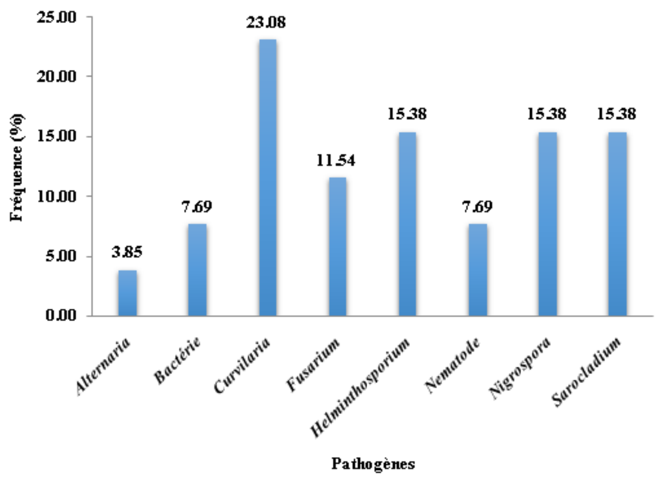


Figure 14: Presence of Pathogens on the Irrigated Perimeter of Zè

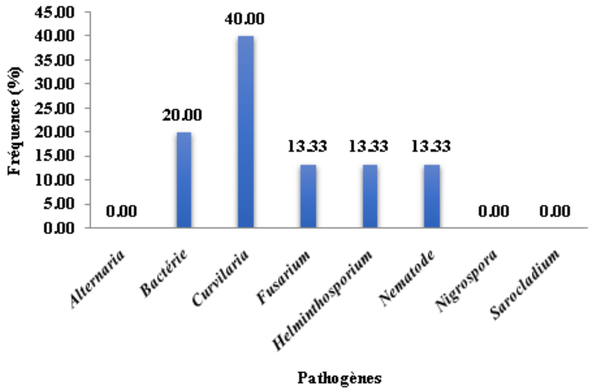


Figure 15: Presence of pathogenic Varieties Collected Africa Rice

Overall, we noted a low severity of attack of production by certain diseases as is the case *Sarocladium oryzae oryzae of Nigrospora* etc. Also 14.63% of production are attacked by the disease of brown spots caused by H *elminthosporium sp,* 29.27% by the disease of seed discoloration caused by *Curvilaria lunata, and 9.76%* for nematodes. The case of the most common diseases is therefore linked to the presence of *Curvilaria lunata and Helminthosporium oryzae,* (Figure 16).

The actual pictures of pathogenic spores observed in microscopic observations are presented in Figure 17.

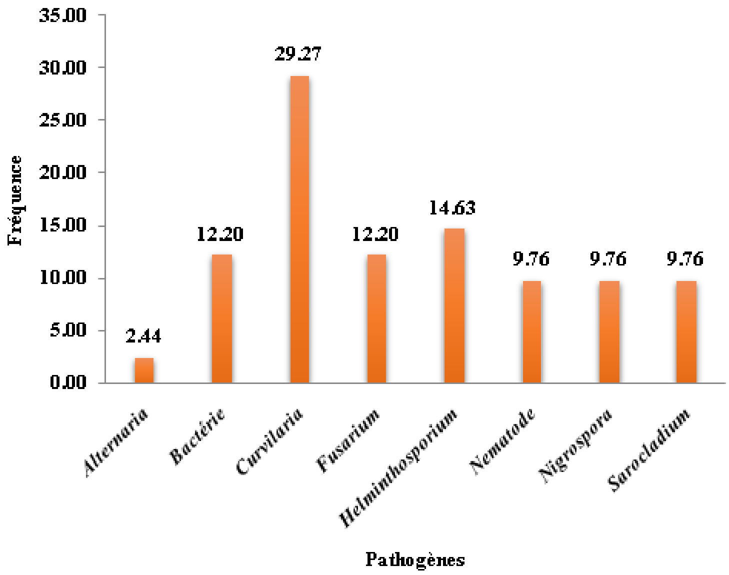
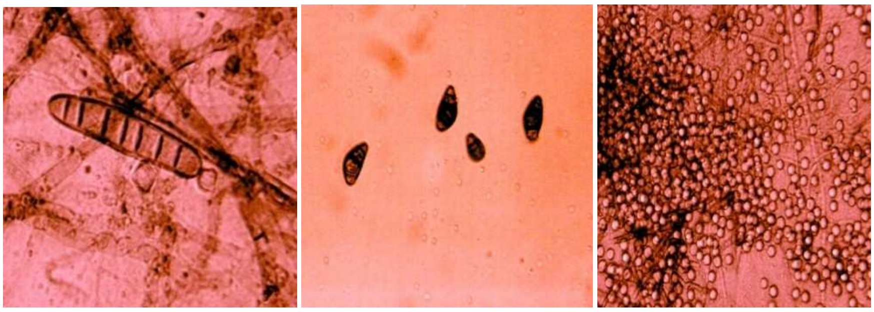


Figure 16: Presence of Pathogens on Both Collection Sites



Nématodes oryzae Helmintosporium oryzae Curvularia oryzae Nigrospora oryzae

Figure 17: Actual Pictures Fungal Spores Observed Under a Microscope Source: HODE, 2014

Table 9: List of Bacteria Isolates Obtained With the Test of KOH

S / N	Sample Code	Nature of the Explant	Biochemical Test
1	ML5-1	Rice seed	Gram-
2	ML5-2	Rice seed	Gram-
3	ML5-3	Rice seed	Gram-
4	ML5-5	Rice seed	Gram-
5	ML5-6	Rice seed	Gram-
6	ML18	Rice seed	Gram-
7	ML19	Rice seed	Gram-
8	ML25-1	Rice seed	Gram-
9	ML25-2	Rice seed	Gram-
10	ML25-3	Rice seed	Gram-

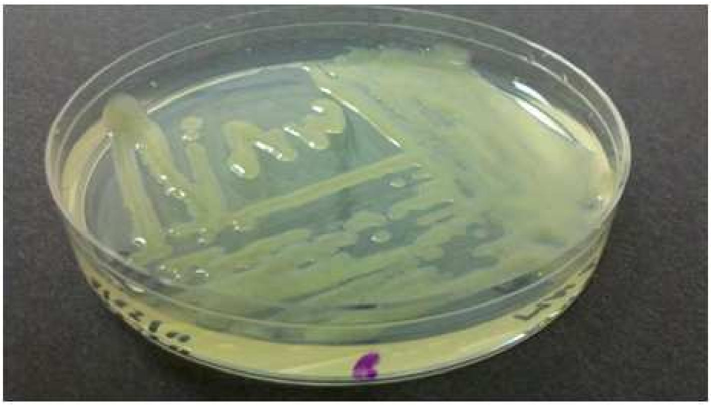


Figure 18: Identification of Bacteria Colonies

Evaluation of Weathering Rates by Fungal Microorganisms

The seed lots treated with the inoculum present a variability of results calculated after the alteration index obtained after three months observation seed (Table 10)

Table 10: Alteration Index and Percentage of Altered Grains

Fungal Species	Index Altering		Percentage of Altered Grains	
	NL 20	IR 64	NL 20	IR 64
Helminthosporium oryzae	0.10 ± 0,001aa	0.11 ± 0,005bb	51.25 ± 1,35aa	76.00 ± 2,05ba
Fusarium moliniforme	0.07 ± 0,005ab	0.09 ± 0,006ba	61.25 ± 2,5bb	59.50 ± 2,1bb
Curvilaria lunata	0.06 ± 0,009ab	0.08 ± 0,009ba	72.00 ± 1,1ac	83.00 ± 1,02bc

The first letters after the numbers are relative to the comparison between the varieties (line) of rice while the second letters are for comparison between fungal species (column). The numbers accompanied by different letters (between row or column) are significantly different (Student-Newman-Keuls)

The evaluation of the disease as a function of time showed that the progression of symptoms observed for the seeds after inoculation varies fungal pathogens strains and varieties tested. The grain infestation is variable over the three months of observation.

For variety NL 20 *H.oryzae*, *C. lunata and F.oryzae* cause alterations whose indices range from 0.06 to 0.1 while these indices vary from 0.08 to 0.11 IR 64.

The *Helminthosporium oryzae* alteration index is significantly different from those of *Fusarium verticiloides* and *Curvilaria lunata* on the variety NL 20. *Helminthosporium oryzae* a 0.1 alteration index which is significantly higher than those of *Fusarium* (0, 07) and *Curvilaria* (0.06). *Helminthosporium oryzae* proves to be more aggressive *and Fusarium* on *Curvilaria* NL 20. This conclusion also applies to the variety IR 64. in fact, on this variety, the index of alteration *H.oryzae of* (0.11) is significantly higher than those of*Fusarium* (0.09) and *Curvilaria* (0.08). By cons, *Fusarium* alteration *of* indices *and Curvilaria* are the same on each variety tested.

The analysis of alteration indexes also shows that the variety NL 20 is more resistant than the variety IR 64. In fact, the pathogenic alteration of indices *(Helminthosporium, Fusarium* and *Curvilaria)* is significantly higher on IR 64 as NL 20.

Considering the evidence of alteration, one can conclude that *Helmintosporium oryzae* is the most aggressive pathogen and the variety NL 20 is more resistant than the variety IR 64.

The percentage of altered grains evolves following types of pathogens and rice varieties tested. On the variety NL 20, altered grain Percentages are significantly different for the three tested pathogens. Curvilaria is the pathogen that most altered grains as *Fusarium and Helminthosporium.* Indeed, 72% of the grains are altered by *Curvilaria* while 61% and 51% are altered by *Fusarium and Helminthosporium* respectively.

On the variety IR64, altered grain percentages are also significantly different for the three tested pathogens. Curvilaria proves to still be the pathogen that most altered grains as *Fusarium and Helminthosporium.* Indeed, 83% of the grains are altered by *Curvilaria* while 59% and 76% are altered by *Fusarium* and *Helminthosporium respectively.*

Analysis of altered grains percentages also shows that the grains of the variety IR-64 are more corrupted than the variety NL 20. However, the grain alteration percentage is not different in the case when *Fusarium* considers the two rice varieties. It should be noted that more than half of the inoculated grains were altered regardless of the pathogen and the variety of rice.

Considering the altered grains percentages, we can conclude that the most aggressive *Curvilaria* pathogen and the variety NL 20 is more resistant than IR64.

DISCUSSIONS

The results of the survey showed that the prevalence of diseases of rice noticed on seed is low on the collection site with a low incidence. The diseases were observed with varying impacts on the site and the Zè AfricaRice. Apart from cases of bacterial diseases simultaneously notes the presence of fungal diseases on the perimeter *caused* by*Nigrospora the Curvilaria the blight, Fusarium* and the False coal. Pathogenic fungi on seeds differ bacterial colonies by the appearance and rapid growth of mycelium. Bacterial colonies are distinguished by their physical aspects gooey, viscous and oily. These characteristics observed in this study on bacteria have been described by (Onasanya et al, 2011). The bacterium was isolated from rice panicle on seeds. These rice panicles are sources of inoculum on the perimeter. This remark made confirms that of Or (1985) which showed that the pathogen can pile up after winter on straw from rice panicles and that this source of inoculum could have importance in areas where bad guest herbs are very poorly developed or non-existent. Among the biochemical tests, we were able to perform on the test isolates KOH 3% to confirm the type of Gram bacteria. We also characterized a kind of nematode whose species is *Aphelenchoides besseyi* (White tip) as a worm.

Indeed the presence of diseases caused by bacteria and fungi *Helminthosporium oryzae, Curvilaria lunata and Fusarium verticiloides* is noted on the samples taken and the observed patches. This variation of incidence may be related to the heterogeneity of environmental conditions such as rainfall as highlighted Sere and Sy (1996) and also by climatic factors.

The alteration of the seed increases when temperature and humidity are favorable to the development of microorganisms (Harison and Perry, 1976; Bothast 1978). The hulls are invaded depth, the caryopsis is covered with brown spots, while the germination of grains decreases (Angladette, 1966). Fungi attacking the grains after harvest, causing in effect, the discoloration of these grains. This discoloration would be due to the high metabolic activity of fungal organisms in some caryopses, either directly or indirectly by one or more species or combinations of various species (Angladette, 1966)

Fungi such as *Helminthosporium oryzae* and *C. lunata* are formidable species causing macular leaf diseases (Bouslin et *al;* 1997 Ennafath et *al.*, 1999; Ouazzani et *al;* 2000; Gnancadja, 2005) but they must also be regarded as serious opponents of seeds.

C. lunata is considered by some authors (Duraiswamy and Mariappan, 1983) as a fungus causing discolouration of grains. This condition is of considerable gravity due to low germination paddy (Querijoro et *al.*, 1993; Jin et *al.*, 1994). Our results are consistent with those developed by Rao and Salam (1954) and Agrawal et *al*.1989 who reported that if the conditions are favorable (65% higher humidity), the degree of rice grains infection can reach 60%.

Fusarium verticiloides is frequently transmitted by seeds (Ould, 1989). This fungus and causes discoloration of rice grains (Bedi and Dhliwal, 1971), the seriously infected kernels are discolored, sometimes turning pink under the effect of the presence of conidial masses (Hino and Furuta, 1968). The presence of *F. verticiloides* can contribute significantly to the reduction of grain quality.

The grain discoloration is associated with impaired quality (Duraiswamy and Marippan, 1983), a reduction in seed viability and dieback of seedlings before or after emergence (Hassikou 2000). Rice Grain fading process is a complex process associated with the expression of various plant pathogens, including fungi. It is easy to note that a number of species such as *F. moniliforme, C. lunata, H. oryzae* can be considered major species involved in the discoloration of the grains. *C.* lunata. *H. oryzae and F. verticillioides*have a real capacity to cause damage on seeds. The damage caused can increase or decrease depending on the conditions of storage of grains.

Although the incidence recorded in some cases is low or low, studies have shown that the small percentage of infection in a seed lot is enough to cause epidemics (Wallen and Sutton 1965). In addition, this indication shows that the seed is true important mode of dissemination of pathological diseases.

CONCLUSIONS

The results of this study reveal that the seeds are true pathogens speculators. Different cases of pathogens are present in most of the seeds grown by farmers. Of the sixteen (16) rice varieties collected seven (07) groups of pathogens were observed namely *C. lunata, H oryzae, N. oryzae, F. verticillioides, A. oryzae,* bacteria and nematodes. Also, we have shown that *H. oryzae* is the most aggressive and pathogenic *C. lunata* that affects more grain.

REFERENCES

1. ADEKAMBI SA, (2005), Impact of the adoption of improved rice varieties on the education and health of children in Benin: case of the Department of hills, engineer agronomist Memory, University of Abomey (Republic of Benin)
2. Adegbola PY and Singbo AG (2005). Impact of imports of rice on the competitiveness and profitability of domestic production in Benin. PAPA / 13p INRAB
3. Adegbola, PY and Sodjinou, E. (2003). Study the competitiveness of rice production systems in Benin by the policy analysis matrix. PAPA / INRAB. 19p
4. Adegbola, PY and Sodjinou, P. (2003). the VCA rice in Benin PADSA / PAPA / INRAB, Porto-Novo, 232p
5. Agbodjalou H. (2009), NERICA production in Benin: Identification of suitable areas by analyzing geospatial 18p
6. Ahouansou R,, Affopkon A., *(2012)* in Benin Rice: improved production technologies and processing and prospects CRA-South / INRA
7. Africa Rice BTF, Meeting Saint Louis, Senegal May 29-June02, (2012) Selection for Resistance to Biotic Stresses of Rice Breeding Training Course Saint Louis, Senegal, Saint Louis, Senegal
8. Albar, L., Baugratz-Reyser, M., Hebrard, E., Ndjiondjop, MN, Jones, M., Guesquière, A. (2006). Mutation in the eIF (iso) 4G translation initiation factor Confers high resistance of rice to RYMV. The Plant Pathology Journal 47, 417-426.
9. Anonymous (1994). Memento l`agronome. Ministry of Cooperation, Paris. 1644p.

10. Anonymous (2002). Memento agronomist, Ministry of Foreign Affairs - CIRAD GRET, pp 799-811 (1687p).

11. Angladette A. (1966). Rice, GP New house Larose. Paris (V ^{e}) P: 609-669. Ann. Rev. Phytopathol; 16: 83-101

12. Arraudeau, MA (1998). Irrigated Rice: The Tropical Agriculture Technician. Maisonneuve and Larosse, Paris. 331 p

13. Bouslin F., B. Ennaffath, Ouazzani Touhami A., Douira A, E and El Haloui N. (1997) Pathogenesis compared to some Moroccan isolates of Helminthosporium oryzae vis -a- vis certain varieties of rice (Oryza sativa). Awamia, 10: 9-18

14. Bothast. RJ (1978). Fungal deterioration and related phenomena in cereals, legumes and oil seeds. Pages 210- 243 in post harvest biology and biotechnology HO Hultin and Milner, eds. Food and Nutrition Press. Inc., Westport. Ct, 462P

15. PS Bedi and Dhhival. DS (1971). Spermatosphere native variety of rice from different states of India. India. Phytopathol. 23: 708-710

16. Chaudhary RC,, JS Nanda and. Tran DV (1998) Identification Guide to the field constraints to rice production: SECTION 3: Problems and obstacles to rice production

17. Christensen CM, (1969) Influence of moisture. happy time and temperature of storage up on invasion of rough rice by fungi. Phytopathology, 145-148

18. Dossa G. S. C (2010) Importance of Bacterial Wilt rice on the Irrigated Perimeter Malanville p77

19. Duraiswamy, VS and Marippan, V. (1983) Rice grain discoloration International Rice Research Newsletter 8: 3

20. Duraiswamy, VS and Marippan, V. (1983) Biochemical prosperity of discolored rice grain. International Rice Research Newsletter8: 3

21. Ennaffath B., A. Ouazzani Thami El Yachioni Mr. Douvia. A. (1999) Pathogenic capacity of Helminthosporium speciferum: floria pest of rice in Morocco. Journal of Phytopathology, 147: 377- 379.

22. Gnancadja. L.,. Ouzzani Touhami A,, A,, Badoc Douira A. (2004), Bull. Soc. Pharm. Bordeaux, 143, 39-50 Detection Test fungal contaminants of rice grain at the end of the vegetative cycle

23. Gnancadja LS, Quazzani Touhani A,., Douira Allal (2005). Effect of certain fungal species on the development of tarnish rice grains Rev Inst Agr. Vet. HassanII. vol: 5

24. Hubert P, (2006) ECR special agricultures datasheets insect Agronomy Engineer of rice in West Africa 1st International Conference on the organic rice production systems, Montpellier, France p52

25. Hino, T, and Furuta, T. (1968) studies on the control of Bakanae disease of rice plants Caused by Gibberella fujikuroi. II. Influence on flowering season on rice plants and seed transmissibility through flower infection Bulletin of the Agricultural Experiment Station chugoku F2: 97110

26. Harison JG Perry DA (1976). Studies on the Mechanisms of barley seed deterioration, Ann Appl Biol,, 84, 57-62

27. Hassikou K. (2000). Contribution to the study of Curvilaria Lunata, the causal agent of rice curvilariose Morocco. Application of some means of chemical and biological control doctoral thesis National, Morocco Faculty of Science, 187 P
28. IRRI International Rice Research Institute-November (2002) Standard Evaluation System for Rice (SES) Find out how the qualities of rice are and evaluate-score in this authoritative sourcebook Los Banos Philippines 56p
29. IRRI-Internationa1 Rice Testing Program (1988) Standard assessment system for rice. 3d ed. International Rice Research Institute, PO Box 933, Manila, Philippines.
30. ISTA-International Seed Testing Association (1985) International Seed Testing Association rule book. Seed Sci. and Technol. 13 (2): 299-520.
31. Jin MZ, Cair Y., Zhang Lin QS & WC (1994) Preliminary study of symptoms and pathogen of colored rice grains. Plant- protection; 20: 7-8
32. Jacquot. And Mr. Courtois. B., (1986) Rice Rainfed by collection led by Rene Coste, tropical agronomy engineer.
33. TW and Mew. Misra JK (1994) A Manual of Rice Seed Health Testing
34. Los Baños, Laguna, Philippines Mail Address: PO Box 933, 1099 Manila, Philippines P 12O
35. Nonfon. Mr. October (2005) Support for husking paddy rice in the Collines Department: the experience of Benin VECO and its partners.
36. Nwilene, E. (1999). Current Status and Management of insect vectors of rice yellow mottle virus in Africa. Insect Sci. Appl. Vol 19, No. 2/3, pp. 179-185.
37. Or, SH (1985). Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey,
38. England. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh - 2202, Bangladesh * Corresponding author and Email: dhossain69@gmail.com 380 pp.
39. Onasanya A. Y Sere. (2011). Two pathotypes of Xanthomonas oryzae pv. Identify oryzae Virulence in West Africa. Africa Rice Center (WARDA), PMB 5320, Ibadan, Nigeria. 14p.
40. Onasanya A. Sere Y. (2009). Two pathotypes of Xanthomonas oryzae pv. Identify oryzae Virulence in West Africa. Africa Rice Center (WARDA), PMB 5320, Ibadan, Nigeria.
41. Ouazzani Touhami A., Ennaffath. B., El Yachioni Mr. Douvia. A. (2000) - Pathogenesis compared to 4 species helminthosporium obtained from diseased plants rice in Morocco. - J. Phytopathol, 148 (4). 221-226.
42. Sidi Ould A, (1989). Contribution to the study of rice seed diseases (Oryzae sativa L) in Morocco, end of study thesis submitted for obtaining an engineering degree from the ENA Meknes. technical and development option, 38p.
43. Querijero M. M, Siso EG & Sevilla EP (1993). Preliminary study of fungi associated with discolored rice seed (Oryzae sativa) and Its effect on germination. Pest Management council of the Philippines, Inc; College, Laguna (Philippines); Department of Agriculture, P.63-65

44. Reversat, G., Bouman, B. Fernandez, L., Lampayan, A. and Castaneda, A. (2003). How water shortage in irrigated Asian rice producing potential threat of plant parasitic nematodes. SPPD-seventh international conference on plant Diseases, Tours, France, December 3-5, 2003. 8p.

45. Savary, S. et al., (2000). Rice pest constraints in tropical Asia: Losses quantification of yield due to pests in rice output has ranks of situations. Plant Dis. 84, 357-369

46. Y Sere Onasanya,, A., K. Akator March 19 (2010) biotic Rice: Identification of rice diseases in Africa Y. y.sere@cgiar.org sere_y@hotmail.com Cotonou

47. Sy, A., and Séré, Y. (1996). Pathology Training Manual WARDA rice. Imprint Design. UK. 94p.

48. Togola A. et al., 2010a. Knowledge of peasant rice stem borer insects and their damage in different ecological zones of Benin (West Africa). Cah. Agric., 19, 262-266

49. Veena, MS, Shetty, HS, Mortensen, CN, and Mathur, SB (2000) Bacterial Leaf Blight of rice. Technical Bulletin. DTP and Offset Printing by Surina Graphics, Mysne. Ph: (0) 516296, (A) 513193.

50. Traore Mahamadou (1987) seed terminal fungi of rice in cote d'Ivoire Their effect on seed germination and control.. A postponement Submitted for the examination of seed pathology Held at the Danish Institute of seed pathology for Developing countries Copenhagen Denmark: 140

51. Wallen, VR and Sutton, MD (1965). Xanthomonas phaseoli var fuscans (Burkh) Starr & Burkh. on field beanin Ontario. Canadian Journal of Botany. 43, 437-446.

52. Wikipedia: Free encyclopedia. Www.wikipedia.fr, accessed 25-02-2009.14p.

53. Yeasmin F,, Ashrafuzzaman Mr. Hossain and I (2O12). Effects of Garlic Extract, Allamanda Leaf Extract and we Provax -200 Seed Borne Fungi of Rice p20. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh - 2202, Bangladesh.