# EFFECT OF DIFFERENT DOSES OF NPK FERTILIZER ON THE INFECTION COEFFICIENT OF RICE (Orysa sativa L.) BLAST IN NDOP, NORTH WEST OF CAMEROON

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#### **ABSTRACT**

The developpement of fungi diseases such as Rice blast which cause important yield losses in rice cultivation in Cameroon is favorite by a variation of doses in minerale fertilisation. Since those elements facilitate the infection process and the development of pathogens. As part of the solutions to this constraint, a field trial was conducted using NPK fertilizer at three different doses, i.e., 180 kg/ha; 200kg/ha; 220 kg/ha plus a control (0 kg/ha). Four rice varieties were used: NERICA 3 and NERICA 7 as upland rice varieties while NERICA 36 and NERICA 42 as lowland rice varieties. To evaluate the influence of the different doses of fertilizers (NPK), a randomised complete block design was used. Disease parameters like incidence and severity of rice blast were evaluated at all the growth stages. Results obtained revealed that disease incidence and severity of rice blast during the vegetative growth of the different varieties of rice, was more significant on the control compared to the other treatments and lowland rice varieties were more infected than upland rice. Fertilizer N-P-K (20-10-10) at the dose 200 kg/ha makes the plant less susceptible to the blast at all its growth phases. Thus, the use of fertilizers at the proper doses is necessary for fighting against rice blast.

Keywords: Rice blast, NPK Fertilizer, rice varieties

#### RESUME

### EFFET DES DIFFERENTES DOSES DE FERTILISANT NPK SUR LE COEFFICIENT D'INFECTION DE LA PYRICULARIOSE DU RIZ (Orysa sativa L.) A NDOP, NORD-OUEST DU CAMEROUN

Le développement des maladies fongiques, telles que la Pyriculariose du riz, qui cause des pertes de rendement importantes en riziculture au Cameroun, est favorisé par une variation dans l'apport des doses de fertilisants NPK. Car ces éléments facilitent le processus d'infection et de développement des pathogènes. En tant qu'élément de solution à cette contrainte, une étude a été menée en utilisant les engrais NPK à trois doses différentes : 180 kg/ha ; 200 kg/ha ; 220 kg/ha et un témoin négatif (0 kg/ha). Quatre variétés de riz de NERICA ont été utilisées : NERICA 3, NERICA 7 (riz pluvial) NERICA 36, NERICA 42 (riz irrigable). Pour évaluer l'influence des différentes doses d'engrais (NPK), un dispositif en blocs complets randomisés a été utilisé en champ. Les paramètres de maladies tels que : l'incidence et la sévérité ont été évalués à tous les stades de développement du riz. Les résultats obtenus ont montré que l'incidence et la sévérité de la pyriculariose du riz, pendant la phase de croissance végétative, étaient plus élevées dans le témoin comparé aux autres traitements et le riz irrigable a le plus été infecté par la maladie comparé au riz pluvial. L'engrais N-P-K (20-10-10), à la dose 200 kg/ha, rend la plante moins susceptible à la pyriculariose, à toutes ses phases de croissance. Ainsi, l'utilisation des engrais, aux doses appropriées, est nécessaire dans lutte contre la pyriculariose.

Mots clés : Pyriculariose du riz, engrais NPK, variétés de riz

#### INTRODUCTION

Rice (Oryza sativa L.) constitutes a staple food for the Cameroon population, and it is the most consumed cereal; indeed 75 % of basic food come from cereal (IRAD, 2013). Between 1961 and 2007, the importation of rice in central Africa region was multiplied by 14; passing from 32 100 to 470 974 tons, whereas the cereal production per habitant regressed from 157 to 84.9 kg. Therefore, the national strategy for the development of rice cultivation (SNDR) was elaborated in response to this problem in Cameroon (Folefack, 2014). Factors such as the insufficient cultivated methods; the poor access to fields; insufficiently trained farmers; the pressure of parasites such as bacteria, fungi, viruses and pest damages (by insects and birds) are responsible for poor yield of rice. Moreover, the bad cultivation methods increase the susceptibility of the plants to the fungi, viral and bacterial diseases (Imrani et al., 2014). These diseases can reduce the output by 10 to 100 % (Ou, 1985). However, the studies carried out by Ben Mohamed et al. (2000) show that correct use of fertilizers increases the resistance of the plants to the diseases. According to Anderson (2002), there would be a close connection between the nutrients status and diseases of plants. The nutrition would play a significant role in the mechanism of defense of the plants (Huber and Jeff, 2012) the mineral nutrition is used as prevention methods against many diseases (Ahangar, 2013). In some cases, it considerably decreases the use of fungicide (Hervieux et al., 2002). In Ndop plain (North-west Cameroon), rice cultivation is the main activity of the population. Former studies on rice in Ndop were focused on : the adequate management of water in lowland rice (Fonteh et al. 2013; Djomo et al., 2017); on the impact of rice cultivation in the economy of the population (Zephania and Nghengwa, 2014) and the effect of plants extracts on rice seed fungi (Nguefack et al., 2013). Several authors work on rice and its diseases. However, little information is available about the influence of the different doses of fertilizers on the diseases and the yield of rice. Therefore, this study was conducted with the objective of evaluating the effect of the different doses of fertilizers N-P-K ((23-10-05) and (20-10-10)) on the infection coefficient of Pyricularia grisea causal agent of rice blast.

### **MATERIALS AND METHODS**

#### STUDY SITE

The study was carried out in Ndop plain, in the experimental plots of the Institut de la Recherche Agricole pour le Developpment (IRAD) and at the Upper Nun Valley Development Authority (UNVDA), Ngoketunjia Division in the North-West region of Cameroon. The town lies between longitude 10° 15' and 10° 50' east, and between latitude 5° 4' and 5° 10' North with an altitude of about 300m. The climate is of equatorial type and is characterized by a raining season (from March to November), and a dry season (from November to February). The annual average temperature is 27.2 °C, and annual average pluviometry is 2500 mm. The rainfall pattern is monomodal. The topography of Ndop is dominated by plains and erosion of volcanic materials from the surrounding mountainous chains, which makes the soil ferralitic and fertile.

#### SETTING UP OF TRIAL

The trial was set up from June to November 2015 in a Randomized Complete Block Design (RCBD) in a manner following conventional methods. The RCBD was use in order to minimize the heterogenicity of the experimental plots. Each block had four treatments: 0 kg/ha 180 kg/ha; 200 kg/ha; 220 kg/ha, replicated three times. 100kg/ha of urea was uniformly applied to all the treatments during tilling and before flowering. These treatments were separated by border rows of 1m wide. Four varieties of rice were used for this study (NERICA 3 NERICA 7 NERICA 36 and NERICA 42). NERICA 3 and NERICA 7 were upland varieties which are less susceptible, and NERICA 36 and NERICA 42 were lowland varieties which are more susceptible. For each variety and the two fertilizers 24 plots of 9 m<sup>2</sup> were used. A total of 48 upland plots were used to plant upland; lowland varieties were plant in 48 irrigate plots in Ndop (North-wester Cameroon). The seeds were planted at 25 × 25 cm apart, and this gave a density of 16 plants per m<sup>2</sup>. Application of fertilizer was done 21 day after planting (DAP). The second application of fertilizer was done at 51 DAP. Weeding was done on emergency, before flowering and one week after flowering. Two types of fertilizers were used: N-P-K (20-10-10) and N-P-K (23-10-05). In any separate trial.

#### DISEASES INCIDENCE EVALUATION

The incidence of the disease were evaluated every week at different development stages which are: germination, vegetative growth and maturity according to this formula below.

Diseases incidence (%) =  $\frac{\text{number of attact plant}}{\text{total number of plant}} \times 100$ 

#### DISEASES SEVERITY EVALUATION

Severity was rated using a modification scoring scale of 0 - 9 described by Notteghem et al. (1980) as follows: 0 = indicates no symptoms, 1 = verry few symptoms, 2 = few lesions corresponding to less than 1 % of the leaf area with symptoms, 3 = several lesions, but not linked together corresponding to 1 - 5 % infected leaf area, 4 = many lesions some linked together to form a necrotic (dead) area corresponding to 6 - 20 % infected leaf area, 5 = necrotic areas linked together and a few leaf tips are dead corresponding to 21 - 50 % infected leaf area, 6 = 50 % of the leaf tips are dead corresponding to more than 50 % leaf area with symptoms, from 7 to 9 = most of the leaves are dead or the plant is dead.

This severity was evaluated at a periodicity of 7 days. Thus, various surfaces of the foliar lesions were evaluated, and according to the importance of the lesion; the scales were allocated there.

#### **DETERMITION OF INFECTION COEFFICIENT**

The infection coefficient was determine by multiplying the incidence by : the severity

Infection coefficient = Incidence X Severity

#### PATHOGENICITY TEST

## Isolation and identification of Pyricularia grisea

Green infected plants rice portions with active lesions were brought into the crop protection laboratory of IRAD Bambui (North-west Cameroon) for isolation of *P. grisea*. These plants rice portions were washed in tap water and cut into small pieces of 2 mm², showing half healthy and half diseased tissue, with the help of a sterile scalpel. The pieces were surface sterilized by immersing in 5 % sodium hypochlorite solution for two minutes followed by rinsing with sterilized distilled water three times. Using sterile forceps, each of the pieces

were then aseptically transferred to Petri dishes containing 20 ml Potatoes Dextrose Agar (PDA) medium amended with Chloramphenicol (1 g/l) to prevent some bacterial contamination. After five days of incubation at  $21 \pm 2\,^{\circ}$ C, the growing mycelium was sub-cultured on fresh PDA medium until obtained pure cultures which were maintained in a refrigerator at  $4\,^{\circ}$ C. The conidial morphology and mycelia structure without strain identification were identified according to the key of mycology (Barnett and Hunter, 1972).

### Preparation of the inoculum

The culture of P. grisea of 14 days old was scraped superficially using a metal spatula in 60 ml of sterilized distilled water then filtered using muslin. 20 drops of tween 80 were added to the suspension then homogenizes using a vortex. The quantification with 2 X 10<sup>4</sup> conidia/ml was performed using a hemocytometer.

### Seedlings preparation for inoculation

The rice seeds of variety NERICA 3, 7, 36 and 42 were disinfected by steeping in a 10 % sodium hypochlorite solution for 10 minutes. Then rinsed twice with a pure water and placed in Petri dish containing a sterile cotton soaked with sterile distilled water. Incubation for germination was made with 23 °C with alternation of 12 hours of light and 12 hours of dark. Seven days after the setting for germination of seeds. The seedlings resulting were plant in a pot containing sterilized ground.

#### Inoculation

The inoculation of the plants was made by spraying 60 ml of conidial suspension on the seedlings. These seedlings were then placed during 48 hours under black and sterile waterplastic covers were sprayed making it possible to maintain a relatively high humidity, necessary to the germination and the direct penetration of the conidia (without wound).

#### DATA ANALYSIS

The collected data on the incidence, severity of diseases and the yield of the different varieties were organized using the excel 2010 spreadsheet (Microsoft office) and imported into the STATGRAPHIC version 16 software for ANOVA as described by Wichura (2006). The Duncan Multiple Range Test (DMRT) at the probability threshold  $\acute{a} = 5$  % was used to determine the differences between the averages.

#### **RESULTS**

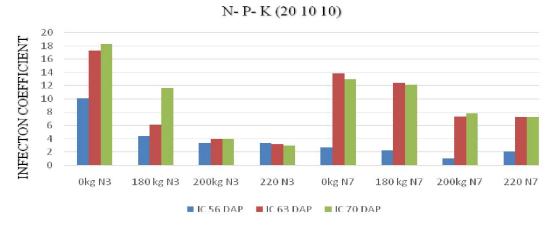
EFFECT OF DIFFERENT DOSES OF FERTILIZER N-P-K ((20-10-10) AND (23-10-05)) ON THE INFECTION COEFFICIENT OF DIFFERENT VARIETIES OF RICE DURING THE VEGETATIVE GROWTH

### Uplands rice: NERICA 3 and NERICA 7

The effect of mineral fertilizers on the infection coefficient of *P. grisea* on the different varieties

of rice is represented by the figures below; the symptoms of disease appear 56 days after planting.

The infection coefficient of upland rice (NERICA 3 and NERICA 7) infected by *P. grisea* was lower than 14 for the plots treated with fertilizer N-P-K (20-10-10) during the vegetative stage compared to the control where the infection coefficient was 18. The smallest infection coefficient (2) noted at the end of vegetative phase was obtained with the doses 220 kg/ha of fertilizer N-P-K (20-10-10) with variety NERICA7 (Figure 1).

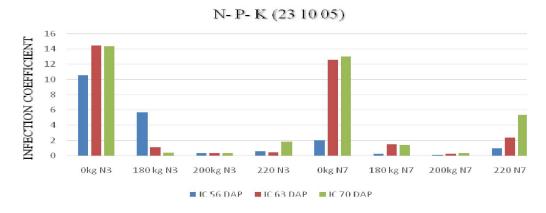


**Figure 1 :** Evolution of infection coefficient of *P. grisea* on upland rice according to different doses of fertilizer N-P-K (20-10-10) DAP, Day After Planting, N 3, NERICA 3; N 7, NERICA 7.

Evolution du coefficient infection de P. grisea sur le riz pluvial en fonction des différentes doses d'engrais N-P-K (20-10-10) N 3, NERICA 3; N 7, NERICA 7.

The infection coefficient of upland rice (NERICA 3 and NERICA 7) infected by *P. grisea* was lower than 6 for the plots treated with fertilizer N-P-K (23-10-05) during the vegetative stage compared to the control where the infection coefficient was

15. The smallest infection coefficient (1) noted at the end of vegetative phase was obtained with the doses 200 kg/ha of fertilizer N-P-K (23-10-05) in all the two varieties (Figure 2).



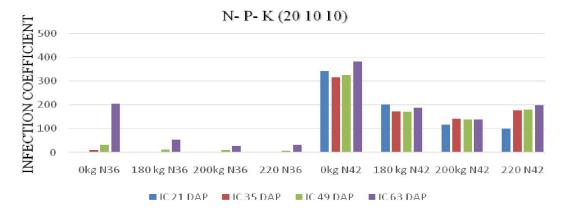
**Figure 2 :** Evolution of infection coefficient of *P. grisea* on upland rice according to different doses of fertilizer N-P-K (23-10-05) DAP, Day After Planting, N 3, NERICA 3; N 7, NERICA 7.

Evolution du coefficient infection de P. grisea sur le riz pluvial en fonction des différentes doses d'engrais N-P-K (23-10-05), N 3, NERICA 3 ; N 7, NERICA 7.

# Lowlands rice: NERICA 36 and NERICA 42

The symptoms of disease appear directly after emergence of lowlands rice. The infection coefficient of NERICA 36 infected by *P. grisea* was lower than 50 for the plots treated with fertilizer N-P-K (20-10-10) during the vegetative stage compared to the control where the

infection coefficient was up to 200. In NERICA 42 the infection coefficient was more than 150 in the treated plots, but in the control plots, the infection coefficient was up to 350. The smallest infection coefficient (25) noted at the end of vegetative phase was obtained with the doses 200kg/ha of fertilizer N-P-K (20-10-10) on variety NERICA 36 (Figure 3).

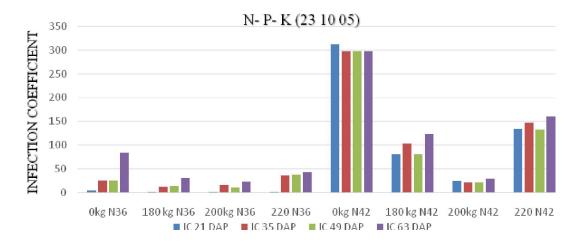


**Figure 3 :** Evolution of infection coefficient of *P. grisea* on upland rice according to different doses of fertilizer N-P-K (20-10-10) DAP, Day After Planting, N 36, NERICA 36; N 42, NERICA 42.

Evolution du coefficient infection de P. grisea sur le riz pluvial en fonction des différente doses d'engrais N-P-K (20-10-10) N 36, NERICA 36 ; N 42, NERICA 42.

The infection coefficient of lowland rice (NERICA 36 and NERICA 42) infected by *P. grisea* was lower than 50 in variety NERICA 36 for the plots treated with fertilizer N-P-K (23-10-05) during the vegetative stage compared to the control where the infection coefficient was 90. In variety N 42

the infection coefficient was more than 50 less than infection coefficient noted in control which was up to 300. The smallest infection coefficient (20) noted at the end of vegetative phase was obtained with the doses 200 kg/ha of fertilizer N-P-K (23-10-05) on variety NERICA 36 (Figure 4).



**Figure 4 :** Evolution of infection coefficient of *P. grisea* on upland rice according to different doses of fertilizer N-P-K (23-10-05) DAP, Day After Planting, N 36, NERICA 36; N 42, NERICA 42.

Evolution du coefficient infection de P. grisea sur le riz pluvial en fonction des différentes doses d'engrais N-P-K (23-10-05), N 36, NERICA 36 ; N 42, NERICA 42.

INFLUENCE OF DIFFERENT DOSES OF FERTILIZER N-P-K ((20-10-10) AND (23-10-05)) ON INFECTION COEFFICIENT OF *P. grisea* ON THE NODES, GRAINS AND THE PANICLES OF DIFFERENT RICE VARIETIES DURING MATURATION

# Uplands rice: NERICA 3 and NERICA 7 NERICA 3

The application of different doses of mineral fertilizer significantly improves the resistance of NERICA 3 to rice blast during the maturation of the grains compared to the control. The plots of NERICA 3 treated with the various doses of fertilizer N-P-K (23-10-05) had an infection coefficient which varied between 3 and 8 % whereas the plots treated with N-P-K (20-10-10) had a infection coefficient which varied between 1 and 4 % while the control had an infection coefficient of 18 % (Table 1).

**Table 1 :** Influence of different doses of fertilizer on the infection coefficient of *P. grisea* on NERICA 3 during the maturation phase.

Influence des différentes doses d'engrais sur lecoefficient infection de P. grisea sur NERICA 3 à la phase de maturation.

Fertilizer	Quantity	Nodes	grains	Panicles
	NERICA3			
	0k g/ha	$8.00 \pm 1.73^{\circ}$	15.67 ± 13.86 <sup>a</sup>	22.00 ± 5.63°
N-P-K (20-10-10)	180 kg/ha	$1.67 \pm 0.33^{a}$	$24.33 \pm 7.88^{a}$	$2.40 \pm 0.67^{b}$
, ,	200 kg/ha	$3.67 \pm 1.60^{b}$	36.33 ± 10.86 <sup>a</sup>	1.00 ± 0.57 <sup>a</sup>
	220 kg/ha	$9.6 \pm 0.88^{c}$	35.33 ± 12.67 <sup>a</sup>	$3.50 \pm 0.67^{b}$
	NERICA3			
	0 kg/ha	$8.67 \pm 2.40^{\circ}$	14.00 ± 2.081 <sup>b</sup>	21.00 ± 2.67°
N-P-K (23-10-05)	180 kg/ha	$2.00 \pm 0.00^{a}$	$4.00 \pm 1.73^{a}$	$3.50 \pm 0.57^{a}$
. ,	200 kg/ha	$3.33 \pm 0.33^{a}$	$4.33 \pm 1.85^{a}$	$7.50 \pm 0.54^{b}$
	220 kg/ha	4.33 ± 1.33 <sup>b</sup>	$4.67 \pm 1.20^{a}$	$7.50 \pm 0.63^{b}$

The values are expressed on average more or less standard deviation. In the same column and for each parameter, the averages which are followed by the same letter are not significantly different with the Duncan test (P < 0.05).

#### **NERICA 7**

The plots of NERICA 7 treated with the different doses of fertilizer N-P-K (23-10-05) had an infection coefficient which varied between 4 and

8 % whereas the plots treated with N-P-K (20 10 10) did not present a significant difference with an infection coefficient of 2.5 % while the control had a coefficient of infection of 19 % (Table 2).

**Table 2 :** Influence of different doses of fertilizer on the infection coefficient of *P. grisea* on NERICA 7 during the maturation phase.

Influence des différentes doses d'engrais sur le coefficient infection de P. grisea sur NERICA 7 à la phase de maturation.

Fertilizer	Quantity	Nodes	grains	Panicles
	NERICA7			
N-P-K (20-10-10)	0k g/ha	9.67 ± 2.40°	14.00 ± 2.081 <sup>b</sup>	19.00 ± 2.43 <sup>b</sup>
	180 kg/ha	$1.00 \pm 0.00^{a}$	5.00 ± 1.73 <sup>a</sup>	$2.67 \pm 0.67^{a}$
	200 kg/ha	$1.33 \pm 0.33^{a}$	5.33 ± 1.85 <sup>a</sup>	$2.00 \pm 0.57^{a}$
	220 kg/ha	3.33 ± 1.33 <sup>b</sup>	5.67 ± 1.20 <sup>a</sup>	$2.66 \pm 0.67^{a}$
	NERICA7			
	0 kg/ha	$9.00 \pm 2.7^{\circ}$	13.67 ± 33.86 <sup>a</sup>	17.50 ±2.56 <sup>d</sup>
N-P-K (23-10-05)	180 kg/ha	$2.67 \pm 0.23^{a}$	26.33 ± 5.88 <sup>b</sup>	$3.66 \pm 0.66^{a}$
	200 kg/ha	$3.67 \pm 0.67^{b}$	$38.43 \pm 9.86^{\circ}$	$5.63 \pm 0.66^{b}$
	220 kg/ha	$8.67 \pm 0.78^{\circ}$	37.43 ± 13.65°	$7.37 \pm 0.66^{bc}$

The values are expressed on average more or less standard deviation. In the same column and for each parameter, the averages which are followed by the same letter are not significantly different with the Duncan test (P < 0.05).

## Lowlands rice: NERICA 36 and NERICA 42

#### **NERICA 36**

The application of different doses of fertilizer increases the resistance of NERICA 36 to the

Rice blast during the maturation of the grains compared to control. The analysis of infection coefficient of *P. grisea* on NERICA 36 treated with fertilizer N-P-K (20-10-10) and N-P-K (23 10 05) did not present a significant difference between the amounts only with the control which had an infection coefficient of more than 8 % (Table 3)

**Table 3**: Influence of different doses of fertilizer on the infection coefficient of *P. grisea* on NERICA 36 during the maturation.

Influence des différentes doses d'engrais sur le coefficient infection de P. grisea sur NERICA 36 à la phase de maturation.

	Quantity	Nodes	grains	Panicles
		NERICA36		
N-P-K (20-10-10)	0 kg/ha	34.33 ± 3.40 <sup>b</sup>	39.00 ± 3.05 <sup>b</sup>	9.00 ± 1.53 <sup>b</sup>
	180 kg/ha	7.00 ± 1.53 <sup>a</sup>	8.66 ± 1.85 <sup>a</sup>	$1.67 \pm 0.33^{a}$
	200 kg/ha	7.33 ± 1.76 <sup>a</sup>	7.67 ± 1.76 <sup>a</sup>	$2.00 \pm 0.00^{a}$
	220 kg/ha	8.33 ± 0.88 <sup>a</sup>	9.33 ± 0.33 <sup>a</sup>	1.67 ± 0.33 <sup>a</sup>
	NERICA36			
N-P-K (23-10-05)	0 kg/ha	35.00 ± 2.88 <sup>b</sup>	38.00 ± 2.88 <sup>b</sup>	$8.00 \pm 1.34^{b}$
	180 kg/ha	$8.00 \pm 0.00^{a}$	$8.33 \pm 0.67^{a}$	$2.33 \pm 0.29^{a}$
	200 kg/ha	$6.00 \pm 1.00^{a}$	$6.33 \pm 0.33^{a}$	$2.67 \pm 0.27^{a}$
	220 kg/ha	$4.00 \pm 0.57^{a}$	$4.67 \pm 0.33^{a}$	$2.33 \pm 0.30^{a}$

The values are expressed on average more or less standard deviation. In the same column and for each parameter, the averages which are followed by the same letter are not significantly different with the Duncan test ( $P \le 0.05$ ).

#### **NERICA 42**

The analyses of infection coefficient of *P. grisea* on NERICA 42 did not show a significant difference between the different doses of fertilizer

N-P-K (20-10-10) and N-P-K (23-10-05) except with the control ( $P \le 0.05$ ) which had an infection coefficient which were more than 10 % whereas the treated plots had infections coefficients of less than 2 % (Table 4)

**Table 4 :** Influence of different doses of fertilizer on the infection coefficient of *P. grisea* on NERICA 42 during the maturation phase.

Influence des différentes doses d'engrais sur le coefficient infection de P. grisea sur NERICA 42 à la phase de maturation.

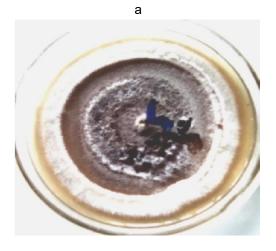
Fertilizer	Quantity	Nodes	grains	Panicles
	NERICA42			
N-P-K (20-10-10)	0 kg/ha	44.67 ± 3.17°	45.67 ± 1.67 <sup>d</sup>	11.00 ± 1.63 <sup>b</sup>
	180 kg/ha	$8.67 \pm 0.66^{b}$	$9.33 \pm 0.66^{\circ}$	1.67 ± 0.67 <sup>a</sup>
	200 kg/ha	$5.66 \pm 0.33^{a}$	$4.66 \pm 0.66^{a}$	$1.50 \pm 0.33^{a}$
	220 kg/ha	$8.00 \pm 0.57^{b}$	$7.67 \pm 0.67^{b}$	1.33 ± 0.33 <sup>a</sup>
	NERICA42			
N-P-K (23-10-05)	0 kg/ha	47.00 ± 1.73°	47.67 ± 1.20°	10.00 ± 1.33 <sup>b</sup>
	180 kg/ha	$7.00 \pm 0.57^{b}$	6.33 ± 1.85 <sup>b</sup>	$2.67 \pm 0.33^{a}$
	200 kg/ha	$7.33 \pm 1.20^{b}$	6.00 ± 1.15 <sup>b</sup>	$2.67 \pm 0.33^{a}$
	220 kg/ha	$4.00 \pm 1.00^{a}$	$3.66 \pm 0.67^{a}$	$2.33 \pm 0.33^{a}$

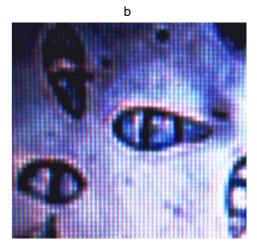
The values are expressed on average more or less standard deviation. In the same column and for each parameter, the averages which are followed by the same letter are not significantly different with the Duncan test ( $P \le 0.05$ ).

# EFFECT OF INOCULATION OF *P. grisea* ISOLATES ON THE DIFFERENT VARIETIES USED

# Description of *P. grisea* isolated on the infected portions of plants rice

The pure culture of *P. grisea* of 14 days old on PDA medium presented cylindrical mycelia with greyish color with cottony aspect consisted of conidiophore. The microscopic observations showed a mycelium of partitioned structure. The conidia had a hyaline structure and equipped with two partitions (Figure 5).





**Figure 5 :** *Pyricularia grisea* ; a : pure culture and b :conidia (x 400). *Pyricularia grisea* ; a: pure culture et b :conidia.

# Reaction of the rice seedlings after inoculation

This test shows that without any fertilization all the varieties tested are infected by *P. grisea*. Lowland rice NERICA 42 and NERICA 36 are more infected compared to upland rice NERICA 3 and NERICA 7 which are less infected. The symptoms appeared as from the fifth day after the inoculation. All the seedlings were infected that led to the drying of the seedling a few days after.

#### DISCUSSION

The effect of the different doses of fertilizers on the blast varies for the four varieties used. The infection coefficient of the P. grisea in varieties NERICA 36 and NERICA 42 reached 350. However with the dose of 180 kg/ha the NERICA 36 treated with N-P-K (20-10-10) had an infection coefficient of 25 and with 200 kg/ha of fertilizer N-P-K (23-10-05) it has an infection coefficient of 26. This would be due to their environment which is always wet. Moreover, fertilizer N-P-K would allow the plants to resist to the biotic stresses such as the fungi diseases. Indeed, the nutritional factors change the chemical composition of the cell by reinforcing their pectocellulosic membranes to prevent the penetration of pathogenic (Ben Mohamed, 2000; Anderson, 2002). Thus, resistance to the fungi diseases depends on the thickness of the cuticle, and skin, the incorporation of silica on the leaf area, thickness, pectocellulosic membrane and opening of the stomata. Potassium intervenes in the balance acid basic of the cells and regularizes the intracellular exchanges, it contributes to reinforce the cellular wall; thus offering to the plants a better resistance to the diseases or to parasites (Doderman, 2000). More there is nitrogen in the medium more the vegetative growth is significant. An increase in the concentration of the amino acids in the apoplast and on the surface of the leaves which induces the germination of the conidia was observed. A great quantity of nitrogen changes the metabolism of the plant by reducing the activity of enzymes responsible for the biosynthesis of phenol and lignin which reduces phenol and lignin in the cell whereas they are involve in the defense system of the vegetable cell against the infections (Hoffland, 1999).

Work of Imrani et al. (2014) reported that

potassium is a major factor in the reduction of the foliar diseases of rice. Indeed, in the case of a lack of potassium the exudates of the plant can contain some components (amino sugar and acids) which would cause the establishment of great fungi infections. The balance between nitrogen and potassium affect the susceptibility of the plant to the diseases. The application of potassium lowers the severity of the fungi diseases and increases the output (Sharna et al., 2005). Potassium also plays a significant role in the development of cuticle which constitutes a significant physiological barrier against infections (Benkinane and Douira, 2014).

At the maturation phase we observed an increase of panicle blast for the plants treated with N-P-K (23-10-05): with the amount 220 kg/ ha that will be due to high nitrogen quantity in manure. According to work of Imrani et al. (2014), the combination of an adequate potassium content accompanied by a phosphorus and nitrogen excess make the plant more sensitive to fungi diseases. Raynal et al. (2014) reports that, nitrogen influences the sensitivity of the tomato and lettuce cultures to the microorganism. In general, when the availability of nitrogen is larger than the capacity of the plant to use it effectively; there is an increase in the infection coefficient of pathogen. Moreover a need of an adequacy between the three major elements of nutrition of the plants is necessary (Huber and Haneklaus, 2007).

The reactions of the varieties used with respect to *P. grisea* were not the same. The results obtained show that the isolates of *P. grisea* are able to deteriorate the plant. For these young seedlings deterioration takes place on the level of the collets and deprives the air part of nutriment and it follows from there a death of the seedling (Puri *et al.*, 2006). This could be due to the fact that during pulverization the pathogenic ones accumulated more on the level of the collet. The most significant varieties died a few days after the appearance of the first symptoms on the air part. However, *P. grisea* was less virulent on the variety upland variety NERICA 3 and more virulent on lowland variety NERICA 42

Castejón-Muñoz (2008) reports that a relative humidity of 95 % and an average temperature between 26 - 27 °C are the optimal conditions for the germination and the infection of the rice plants by the spores of *P. grisea*. This confirms the fact that in field lowland rice were more infected than upland rice. The withering and the

death of the seedlings few times after the appearance of the symptoms would be due to the production of toxins by *P. grisea* which led to the changes such as the disturbance of the metabolism of phenols, the activation of the polyphenoloxidases, the deterioration of the pectocellulosic membrane which contributes to the loss of turgor then with died of cell (Mousanejad *et al.*, 2010; Ganesh *et al.*, 2012). Moreover, youthful cells are sensitive to pathogenic because they produce enough of carbohydrate for their growths; those substances constitute the main nutriments of the pathogens (Cumagun *et al.*, 2008).

#### CONCLUSION

This study relative to the influence of different doses of fertilizer NPK on the rice blast contributes to the comprehension of necessity reasonable uses of fertilizer which is an important value in rice cultivation. Thus, the resistance of the plant to the diseases is controlled through the physiological and biochemical processes in relation to the nutritional statute of the plant or the pathogenic one. The nutritional state of the plant influences on its histology and directs the functions of its tissues with respect to the pathogen. The virulence of pathogen and its ability to survive are conditioned by several nutriments. The infection coefficient of P. grisea can decrease with an adequate nutrition of the plant. All the doses of fertilizers used considerably lowered the infection coefficient of the different varieties of rice used. Fertilizer N-P-K (20-10-10) with at the dose of 200 kg/ha makes the plant less sensitive to rice blast at all its phases of growth and improves the output compared with N-P-K (23-10-05). Fertilizer N-P-K (23-10-05) at the maturation phase influences the panicle rice blast by making the plant susceptible and depreciates the quality of the production, as well for lowland rice as upland rice.

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