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Efficacy of Aqueous Extracts of *Azadirachta indica* A. Juss. and *Cymbopogon citratus* (DC) Stapf. Against *Phoma sorghina* (Sacc.) Boerema Dorenbosch and Van Kestereh, According to its Location on the Sorghum Seeds

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Abstract: The location of *P. sorghina* on the different parts of sorghum seed and the efficacy of plant aqueous extract of *C. citratus* and *A. indica* were studied using blotter method. The identification of different parts of sorghum seed under a compound microscope shows that *P. sorghina* is present on all the parts of sorghum seed. There is a positive correlation between the infection of pericarp and the other parts of sorghum seed. The results reveal that 63% of endosperm infection by *P. sorghina* is explained by the infection of pericarp and 58% of embryo infection by *P. sorghina* is explained by the infection of pericarp and endosperm. The use of plant aqueous extracts in seed treatment lowers the infection rate of *P. sorghina* in all the components of sorghum seed. In comparison with the fungicide calthio C., plant aqueous extracts of lemon grass and *A. indica* have a tendency to lower the infection rate of *P. sorghina*.

Key words: *Phoma Sorghina* · Location · Sorghum Seed · Aqueous Extracts · Efficacy

INTRODUCTION

On the surface and internally, seed can carry bacteria, viruses, fungi and other organisms which introduce diseases into previously uninfected areas and countries. Seed treatment constitutes a method use to protect plants in order to increase yield. It is consists of the application of biological agent, physical process and chemical method to a seed lot to protect plants and to favour establishment of healthy culture [1]. In Burkina Faso, fungicides are used as plant and seed protectants.

In Burkina Faso, sorghum is the main culture, in 2009-2010 the area used to its production is 1 553 115 ha and at the same season, the sorghum is produced 1 521 468 t [2]. The production of sorghum encountered some problems, one of which was the presence of a large number of fungi detected on this plant.

P. sorghina is one of the seed-borne fungi most frequently detected on sorghum samples collected in farmers' yards in Burkina Faso [3, 4]. It is one of the fungi responsible of sorghum seed mould. To control this fungus by seed treatment, [3] has shown that plant aqueous extracts of *Cymbopogon citratus* (D. C.) Stapf. and *Azadirachta indica* A. Juss. are efficient in seed treatment against *P. sorghina* and *Colletotrichum graminicola* (Ces.) Wilson.

Evaluation of fungi on vegetative organs of sorghum plants coming from seed treated with aqueous extracts of *C. citratus* and *A. indica* reveals the presence of *P. sorghina* on some leaves, stalks and roots. This result poses the problem of where *P. sorghina* is located on sorghum seed. The Seed treatment method shows that plant aqueous extracts remove *P. sorghina* from the surface of sorghum seed when it is soaked in extract. In order to choose a suitable method to control *P. sorghina* on sorghum seed we have undertaken to study the localization of *P. sorghina* on sorghum seed and the efficacy of aqueous extract in getting rid of *P. sorghina* in the parts of sorghum seed.

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MATERIALS AND METHODS

Sorghum Seed Samples: Sorghum seed samples are identified according to their infection level by *P. sorghina*. On this basis three samples of 1341 So07 infected by *P. sorghina* at the levels 20%, 50 and 70% and the sorghum varieties samples of ICSV 1001 at 15% and sariaso 02 at 35% are used for the test.

Vegetal Plant Species

Cymbopogon Citratus: (D.C.) Stapf. is an aromatic graminaceous of the family of *Poaceae*. *C. citratus* is an inveterate grass which is cultivated for its medicinal properties and its essential oils used in cosmetic and perfumery.

Azadirachta Indica: A. Juss. belongs to the family of the *Meliaceae.* It is a tree whose height reaches 5-20 m. *A. indica* is used for its shade, the wood for heating and its oil which is used to make soap. *A. indica* is also used in medicine and studies show that it has interesting properties for the control of viruses, bacteria, insects and fungi.

Fungicidal Product Use: The chemical product used is Calthio C. It is a fungicide and insecticide which is composed of 25 % chlorpyrifos-ethyl plus 25 % Thirame, WS. The product is used at 20 g of powder for 5 Kg of seed.

Seed Treated with Water: A sampling is done on each sorghum seed sample using a conical divider in order to obtain 100 seeds representative of the sorghum seed sample. These seed are soak separately in Petri dishes containing 7-8 ml of sterile water and incubated for 24 hours at 28-32°C in the laboratory to favour the separation of the different parts of the sorghum seed.

Seed Treated with Fungicide: Seed sampling is done as previously described and 100 sorghum seeds are obtained for the sorghum sample. These seeds are weighed and according to seed weight the suitable quantity of product is measured. The seeds are coated with calthio C. and kept for 24 hours at 28-32°C. After that the seed are soaked separately in Petri dishes containing 7-8 ml of sterile water for 24 hours.

Seed Treated with Plant Aqueous Extracts: Blades of lemon grass dried in the shade are provided by the laboratory Phytofla of Dr. Dakuyo of Banfora. Dried blades of lemon grass are cut into small pieces and crushed in order obtain lemon grass powder. Thirty grams of lemon grass powder are macerated in 100 ml of sterile water. The mixture is kept for 24 hours in the laboratory at 23°C. At the end of the maceration time, aqueous extract is obtained by pressing and filtering the mixture through cheesecloth. The aqueous extract obtained is sterilized at 120°C for 30 minutes.

The aqueous extract of *A. indica* used is supplied by Dr. Nébié of IRSAT/Ouagadougou. This aqueous extract is obtained after an extraction process using water 25% and ethanol 75%. That extract is considered to be concentrated at 100%. To obtain an extract at 10%, 10 ml of the concentrated extract is mixed with 90 ml of sterile water.

After sampling, 100 sorghum seeds are introduced separately into small sterilized test tubes containing 5 ml of each extract. The seed are soaked in aqueous extract for 24 hours at 28°C. At the end of the soaking time seed are plating in Petri dishes containing three moistening blotters discs and incubated in the dark at 28°C in the laboratory.

Dissection and Incubation of the Different Components of Sorghum Seed: At the end of the soaking time, the different parts of the sorghum seed (pericarp, endosperm and embryo) are separated using a scalpel and a holder. From one component to another the material used is sterilized with alcohol then at flame. The different parts of the sorghum seed are plating separately in Petri dishes containing three moistening discs of blotters. The Petri dishes are incubated under 12 hours alternating cycles of near ultraviolet (NUV) and darkness at 22°C for seven days.

All the experiment have taken place in the laboratory of phytopathology of INERA (Institute of Environment and Agricultural Researches) Farakobâ in West Burkina Faso during february-april 2010.

Evaluation: After incubation, the Petri dishes are brought into the laboratory for examination. Each part of the sorghum seed is examined under a compound microscope in order to identify fungi developing on the different parts of sorghum seed. The results are recorded in the seed health report.

Data Analysis and Expression of Results: Data are first recorded in Microsoft Excel office then, they are

statistically analyzed using one-way analysis of variance (ANOVA) when there are significant differences between treatments, means are separated with a multiple classification of Duncan at 5%. In addition, we do a correlation test of Pearson to understand the relation between the infection rates of the different parts of sorghum seed. The SPSS 10.0.5 software is used for all these operations. The results are recorded on tables or in figures.

RESULTS

Evaluation of *P. sorghina* infection rate in the different parts of sorghum seed of samples 1341So07, ICSV 1001 and sariaso 02.

Variance analysis reveals high significant differences between the different treatments at 20%, 50%, 70%, 15% and 35% in sorghum seed naturally infected by *P. sorghina*. The fungus is detected in all the parts of the sorghum seed, whatever the naturally infected seed rate was. In general, for all the extracts, *P. sorghina* infection level is higher on pericarp compared to two other parts (Table 1). The examination of the different parts of sorghum seed after treatment indicates that aqueous extracts of lemon grass and *A. indica* are effective in reducing the rate of *P. sorghina* infection. At a lower rate of infection (20%) the effectiveness of aqueous extracts is not easily perceived (Table 1). Our work also shows that in all the parts of the sorghum seed naturally infected by *P. sorghina*, aqueous extract lowers the infection rate of the fungus compared to the fungicide except on pericarp of ICSV 1001 (Table 1).

Correlation of *P. sorghina* Infection Rate among the Different Components of the Sorghum Seed

Data analysis shows the existence of a high correlation among infection rate of pericarp and the other parts of sorghum seed (endosperm, r = 0,794, p = 0,000; embryo, r = =0,763, p = 0,000). In addition, the presence of *P. sorghina* in albumen has a high correlation with embryo infection by the same fungus (r = 0,759, p = 0,000). The correlation curve among the infection rate of the different parts of sorghum by *P. sorghina* has given coefficients of determination between 0,58 and 0,63 (Figures 1, 2 and 3). The results are indicated on the graphs show that 63% of endosperm infection by *P. sorghina* is explained by the infection of pericarp (Figure 1) and 58% of embryo infection by *P. sorghina* is explained by the pericarp infection (Figure 2). In the same line of thought 58% of embryo infection by *P. sorghina* is explained by the infection of endosperm (Figure 3).

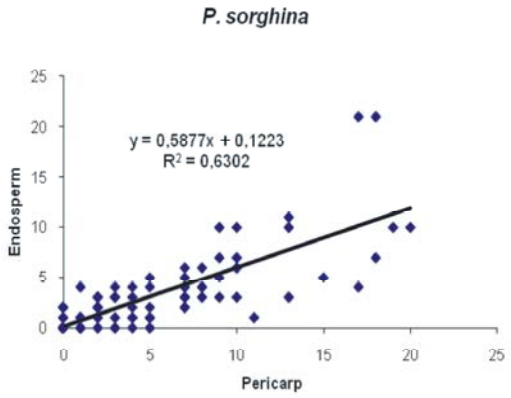

Fig. 1: Correlation curve of *P. sorghina* infection rate between pericarp and endosperm

Table 1: Efficacy of aqueous extracts against *P. sorghina* according to it localization on sorghum seed.

Treatments	*P. sorghina 1341So07* 20%	*P. sorghina 1341So07* 50%	*P. sorghina 1341So07* 70%	*P. sorghina* ICSV 1001	*P. sorghina* Sariso 02
Tte	4,25c	12,5f	17,75g	4,75b	9,5c
Ate	1,5ab	8,5e	14fg	0,25a	1,25a
Ete	0,5a	6cde	9,75ef	0a	0a
Ttf	1,25ab	8,25de	8,75def	0,25a	1,75ab
Atf	1ab	4,25abcd	5,75abcde	1a	1a
Etf	0,25a	1,25ab	2,75abcd	4,5b	3,5b
Tcc	0a	2,75abc	6,75abcde	0,5a	1,25a
Acc	0a	0,25a	4,75abcde	0a	0a
Ecc	0a	1a	0,75a	0,75a	1a
Tai	1ab	3,5abc	7,25bcde	0,75a	1a
Aai	0a	2,5abc	2,25abc	1a	1,5a
Eai	0a	2,75abc	0,75a	0,25a	0,25a

T: Pericarp, A: endosperm, E: embryo; te: Seed soaked in water; tf: Seed coated with fungicide; cc: *Cymbopogon citratus*; ai: *Azadirachta indica*, The numbers follow by the same letter in the same column are not different at 5% according to the multiple classification of Duncan

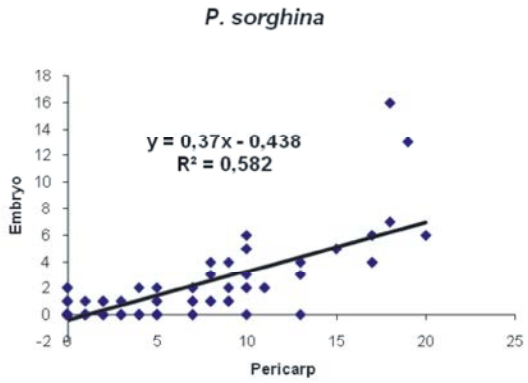

Fig. 2: Correlation curve of *P. sorghina* infection rate between pericarp and embryo

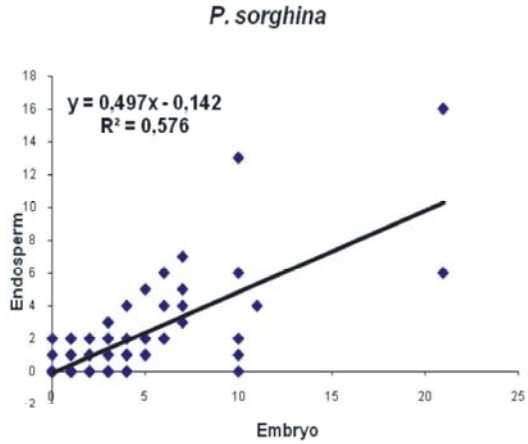

Fig. 3: Correlation curved of *P. sorghina* infection rate between embryo and endosperm

DISCUSSION

The results of the analysis show that *P. sorghina* can infect all the components of sorghum seed [5] noted the presence of *F. moniliforme* on the different parts of sorghum seed. They also found Phoma spp. on the pericarp and on the endosperm of sorghum seed. The infection rate of *P. sorghina* progressively lowers from pericarp to endosperm, then to embryo for all the samples and for all the treatments. *P. sorghina* infection first begins on the pericarp and from there it spreads to the endosperm and to the embryo. The deeper a part is located, the less it is infected by *P. sorghina*. Our results are in agreement with those of [6] who has worked on the location of *Macrophomina phaseolina* (Tassi) Goid. in *vigna unguiculata* L. seed. He shows that the infection rate of the fungus is higher on the pericarp that on the cotyledon and the embryo [7]. in histopathological studies of *Ascochyta fabae* F. sp. *lentis* in lentil seeds, points out clearly that the hilar region of the seed is the first to become infected and from there it spreads to the cotyledon and the embryonal axis.

The study of the correlation of *P. sorghina* infection rate among the different parts of sorghum seed reveals the existence of a positive correlation between these parts. Experience shows that the infection of the pericarp by *P. sorghina* presages the presence of *P. sorghina* in the endosperm and the embryo.

The examination of different parts of the sorghum seed after treatment with an aqueous extract shows that the extracts of lemon grass and *A. indica* are effective in reducing the *P. sorghina* infection rate. Compared with chemical fungicide, the aqueous extracts used are more efficient in reducing the *P. sorghina* rate in naturally infected seed at 50% and 70%.

The components of aqueous extracts dissolved in water are absorbed by the different parts of the seeds when they are soaked in extract. Thus, the aqueous extract should control the fungus in the seed parts which are deeply situated from the pericarp. The fungicide calthio C. can only control fungi on the seed surface and the fungi on the endosperm and the embryo should be transmitted to plant, it can continue to destroy the seed.

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