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EFFICACY AND INDUCTION RESISTANCE OF NEEM EXTRACT AND MANCOZEB 80 WP ON PHYSODERMA BROWN SPOT AND PHYSODERMA STALK ROT DISEASES OF CORN (*ZEA MAYS*) CAUSED BY *PHYSODERMA MAYDIS* IN FAR NORTH CAMEROON

^aNgoh D. J. Patrice, ^aDjongnang Gabriel, ^{b,c}Tene T. P. Martial, ^aTenyang Noel, ^aDeurnaye Placide, ^aMbou T. P. Rony, ^dYamagui Rita, ^dNtatsinda C. Durel, ^eDjile Bouba, ^fAmbang Zachee

^a Department of Biological Sciences, Faculty of Science, University of Maroua, Maroua, Cameroon.

^b Laboratory of Phytoprotection & Plant Valorization, Biotechnology Center, University of Yaoundé I, Messa, Cameroon.

^c Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaounde, Cameroon.

^d Higher National Polytechnic School of Maroua, University of Maroua, PO Box 1450 Maroua, Cameroon.

^e Department of Plants Genetic & Biotechnology, Institute of Agricultural Research for Development of Maroua, Cameroon.

^f Laboratory of Biotechnologies, Phytopathology and Microbiology Unit, University of Yaounde I, Cameroon.

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ABSTRACT

Corn (*Zea mays*) is one of the most widely spread cereals in the world. However, in the Far North Region of Cameroon, the incidence of some maize diseases progress because of farming practices and climate change (rainfall). This work aims to evaluate effect and resistance stimulation of neem extract and Mancozeb fungicide on Brown Spot (PBS) and Stalk Rot (PSR) diseases of corn due to *Physoderma maydis* in field condition. The experimental design was a two-factor split-plot. The treatments were a combination of Control (T), Mancozeb (Mz) at the concentration of 6g/L and aqueous extract of neem seeds (AENS) at the concentration of 50g/L with CMS 9015 and CMS 8704 varieties. Incidence, severity, rainfall, Area Under Disease Progress Curve (AUIPC, AUSiPC) were recorded. Phenol and protein rates, enzymatic activity of Polyphenol oxidase (PPO), peroxidase (POX) and glucanase were carried out. Stalk length and yield were evaluated. Incidence and severity of *Physoderma* Brown Spot (PBS) and *Physoderma* Stalk Rot (PSR) increased with time and environmental conditions (rainfall). The highest disease incidence was observed in the control treatments with both diseases, 52.39 and 37.67 % respectively for Brown Spot and Stalk Rot in CMS8704 variety and lower with AENS, 13.5 and 36.01%. AENS reduced PBS incidence and severity by 23.1 and 19.9 %, respectively and Mz by 9.3 and 18.1 %. The AUIPC and AUSiPC of PBS and PSR remained lower with AENS treatment during the growing season and higher with the other treatments. The highest phenol and proteins rates (8.81 and 25.1 mg/g/FM) were recorded for the AENS treatment in CMS9015 and CMS8704 variety, respectively. Enzymatic activity was more enhanced in CMS8704 variety with AENS. 4.22 A470/min/g, 1.55 A470/min/g and 1.15 μ mole/min /g were recorded respectively for PPO, POX, and Glucane activities. Stalk length was higher in AENS and Mancozeb treatments with variety CMS8704 (125.13 and 123 cm respectively). A yield increase of 7.44% was obtained in the AENS treatment. The combination or not of aqueous neem extract and CMS8704 could be used in the integrated control of PBS and PSR of maize.

Corresponding Author: Ngoh D. J. Patrice

Email: ndjuliopat@yahoo.fr

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INTRODUCTION

Maize is the most cultivated plant and the first cereal produced after wheat and rice in the world (Li *et al.*, 2018). This crop has high economic potential whether it is well managed (Rouanet, 1984). Maize is mainly cultivated for food, animal feed, and as a raw material for many industrial outputs (FAO, 2019).

According to (FAO, 2019), the world maize production from 2018 to-2019 was 1,124 million tonnes. The United States, China, Brazil, and Argentina were among the countries producing about two-thirds of global production. South Africa is the leading maize producing country in Africa. Africa accounts for almost 6.5% of global maize production. Most of the maize is produced during the rainy season. However, it is also grown in the drought conditions in Africa.

Cameroon is the thirty-fifth corn-producing country globally, with 2.100 thousand tons in 2020 (FAO, 2020). This production could not satisfy national demand, which stood at a little over 2.8 million tonnes. Thus, the deficit recorded is more than 500,000 tonnes (MINADER, 2019). Despite the government support program, there is a discrepancy between the needs of industries (maize constitutes up to 80 % of the main chicken feed) and population needs. This deficit is more marked in the West and North of the country, where maize is used in various culinary compositions (Régine and Coderre, 2000). In the Far North Region of Cameroon, this deficit is due to poor farming practices by farmers, inability to buy fertilizers, lack of land, biotic constraints (diseases and pests, choice of seed varieties) and abiotic constraints (climate change, scarcity of rainfall, soil poverty) (MINADER, 2019).

To solve this problem, corn growers are looking for a sustainable alternative by using green and organic fertilizers involving animal dung (chicken droppings and cow dung) to promote microbial activity and soil fertility in the soil (Reid *et al.*, 2001; Veresoglou *et al.*, 2012). Such as animal dung because many farmers combine animal husbandry with crop production. However, other work has also shown that these organic fertilizers (cow dung) also promote and maintain the development of microorganisms that are harmful to crops (Tompkins *et al.*, 1992; Veresoglou *et al.*, 2012).

Research reports indicate diseases due to fungi or bacteria or have been reported in Cameroon on maize, affecting its quantity and quality (William Norbert *et al.*, 2018; Mboussi *et al.*, 2016; Ngoh dooh *et al.*, 2019).

Apart from streak disease, which is the most widespread viral disease, Physoderma Brown Spot appears to be the most common fungal disease during the cropping seasons. Ngoh dooh *et al.* (2019) have shown that brown spot of leaves and stalk rot of maize were caused by *Physoderma maydis* in soil amended with organic fertilizers in the Far North Cameroon.

As in the rest of the world, *Physoderma maydis* is responsible for Physoderma Brown Spot and Stalk rot of corn in the Far North Region of Cameroon (Harvey *et al.*, 1955; Robertson *et al.*, 2015). These diseases lead to a reduction of leaf area and stalk breakage (Tisdale, 1919). In 2013, the estimated yield loss for this disease was more than 13 million plants in the United States and Canada (Mueller and Wise, 2016). The disease frequency in the field is reported to be 80% in southern Iowa of the United States of Cameroon (Robertson *et al.*, 2015).

However, currently, there is a lack of studies and low reported impact of these diseases globally. Moreover, there is not common universal control method (Jakson, 2018). Nevertheless, some methods are adopted against these pathologies. Reduction of inoculum (sporangia) by crop rotation or tillage (Tisdale, 1919), planting adapted and tolerant varieties (Harvey *et al.*, 1955; Mueller and Wise, 2016), use of tebuconazole (Li *et al.*, 2010). However, There is not efficacy and public data on labelled fungicides (despite some reported fungicides) to manage *P. maydis* in the world (Robertson *et al.*, 2015). Researchers are thus necessary to determine the application timing of fungicide to manage these diseases. Although fungicides, unfortunately, have consequences on the environment, human health, and the resistance of microorganisms (Ghormade *et al.*, 2011; Ahmad and Arif, 2010), Finding alternatives to these synthetic products is necessary. The poverty of the population also implies a less expensive method of control for farmers.

Many several studies have already reported induced resistance of plant extracts or their derivatives (Bhuvaneshwari and Paul, 2012; Eloff and McGaw, 2014; Bektas and Eulgem, 2015; Goel and Paul, 2015; Shuping, 2016) to enhanced production of antifungal compounds and other many enzymes to focus management of pathogen fungi. But, no study was undertaken to evaluate the potential inducer of neem seed against *P. maydis*. On the other hand, some chemical products have been reported to induce resistance of plants against fungi (Thakur and Sohal, 2013; William Norbert *et al.*, 2018).

No study currently shows efficacy and resistance

induction of neem extract to PBS and PSR globally, particularly in Africa and Cameroon. In addition, the lengthening rainy season (from 3 to 4 months) in recent years in the Far North zone is a favorable condition for the development of this disease (Burns and Shurtleff, 1973). Aqueous neem extracts effectively against several pathogens that attack crops (Pohe and Agneroh, 2013; Mboussi *et al.*, 2016; Goel *et al.*, 2016). Therefore, this work aims to evaluate the efficiency and reduction induction of neem extract and Mancozeb 80 WP to manage Physoderma Brown Spot (PSB) and Physoderma Stalk Rot (PSR) of corn to *Physoderma maydis* in Far North Cameroon in field condition.

MATERIALS AND METHODS

Study site and plant material

This study was carried out in Pitoare located in the districts of Maroua, Department of Diamaré, Far North Region of Cameroon. The temperature ranged from 28° 40°C. The relative humidity is 80 % in the rainy season and 10-40 %, sometimes in the dry season. Corn has been cultivated in this field for ten years without rotation. During the dry season, oxen, goats and sheep camp in this site at night to improve fertility using the droppings of these animals.

The two varieties of maize, CMS 8704 and CMS 9015 were purchased in IRAD Maroua. CMS 8704 is yellow and has 110-115 days of cycle duration. CMS 9015 is white in color and has 90 days of cycle duration. Both varieties are resistant to *Striga hermonthica*.

Experimental design

The experimental design was a two-factor split-plot consisting of three fully randomized blocks. Each block consisted of six (6) plots (combination of treatments and varieties) for 18 combinations. The plots and blocks were spaced by 1.5 m. Each plot was 4 m long and 3.5 m wide, i.e., an area of 14m² and 84 m² per block. The field had an area of about 300 m².

The corn spacing was 80×40 cm. The planting depth of the corn was about 1.5 cm, with two seeds per pack. About 40 plants were used per plot (variety) and 240 per block. Insecticide CALTHIO (Chlorpyrifos-éthyl ...25%+Thiram...25%) was used to treat seeds against pests.

The different combinations were:

CMS9015-C: CMS9015 untreated (control);

CMS9015-Mz: CMS9015 treated with Mancozeb 80 WP;
CMS9015-AENS: CMS9015 treated with aqueous extract of neem seed;

CMS8704-C: CMS8704 untreated (control);

CMS8704-Mz: CMS8704 treated with Mancozeb 80 WP;

CMS8704-AENS: CMS9015 treated with aqueous extract of neem seed.

Preparation of aqueous extract of neem seeds and Mancozeb concentrations

Aqueous extracts of neem seeds (AENS) were obtained following the protocol described by Ayékpa *et al.* (2010). Neem fruits collected from the foot of the trees were stored for one month at room temperature. Three days before each use, the fruits were soaked in water for 10 hours to separate the pulp and seeds. The seeds were dried in the open air for 24 hours. The seeds were crushed in a mortar. The resulting powder was weighed using a SF-400A scale. Then 800 g were wrapped in a muslin cloth and put in a container containing 10L of water. After 24 hours of maceration, the juice was collected and placed in a 16 L sprayer and then filled to the mark with sterile water. The volume was adjusted for a dose of 50 g/L. The wetting agent (soap) was added to the macerate (1ml /L).

Mancozeb 80 WP, a broad-spectrum, multi-site, and contact fungicide, was used. The recommended concentration of 5-6g/L was prepared. 96 g of Mancozeb was introduced in a sprayer of 16 L.

Application of treatments

Using a 16L capacity knapsack sprayer with an adjustable flow rate, the application of the AENS and Mancozeb 80 WP treatments was localized on the leaves and stalks of each corn variety. Mancozeb was applied once a month and AENS twice a month. The volumes of water and weight of raw material used were adjusted according to the developmental stages of the plants to maintain initial concentration (50 g/L and 6g/L). The application of the treatments began before the appearance of symptoms of diseases in the field.

Assessment of efficacy of treatment on diseases (PSB and PSR) development

Assessment of efficacy of treatment on disease incidences

The incidence of the disease was evaluated every seven days. The incidence was assessed from the 14th day

after sowing. Incidence of diseases was evaluated using the following formula:

$$I (\%) = \frac{n \times 100}{N}$$

Where I is incidence; n number of plants showing symptoms per plot; and N total number of plots.

Assessment of the severity of both diseases

The severity of the disease on infected plants in the field was assessed using a visual rating scale (score) ranging from 0 to 4. The number of leaves attacked by brown spot disease and the number of infected internodes and nodes by stalk rot were counted. The following index score was used:

0 = no symptoms;

1 = 25% :] 0- 1/3] of leaves or internodes infected;

2 = 50% :] 1/4-2/4] of leaves or internodes infected;

3 = 75% :] 2/4-3/4] of leaves or internodes infected;

4 = 100% :] 3/4 -4/4] of leaves or internodes infected.

The disease severity index was calculated according to the formula:

$S = (\sum(x_i \times n_i)) / N \times 100$; where x_i : is the i score of the disease; n_i : the number of plants with i score; N : the total number of diseased plants assessed per plot.

AUDPC "area under disease progress curve"

The Area Under Disease Progress curve (AUDPC) was used to compare the efficacy of AENS and Mancozeb and the evolution of brown leaf spot and stem rot on each of the two varieties. The formulae described by Jager et al. (2001) and Menguala *et al.* (2013) were used to calculate AUIPC (Area Under Disease Incidence Progress Curve) and AUSiPC (Area Under Disease Severity index Progress Curve).

$$AUDPC = \sum_{i=1}^n (X_i + (X_i + 1)/2) (t_2 - t_1)$$

X_i is the incidence of disease at the time i , (t_1) $X_i + 1$ disease incidence recorded at the time $i + 1$ (t_2), n is the number of registration on the incidence, and t , days between the registration of X_i and $X_i + 1$.

Rainfall

The evaluation of rainfall provides information on the degree of precipitation that can influence crop planting and disease progress. The data were collected using rain gauges installed in the middle of the fields. Data were recorded each morning.

Assessment of resistance induction of treatments on two both varieties

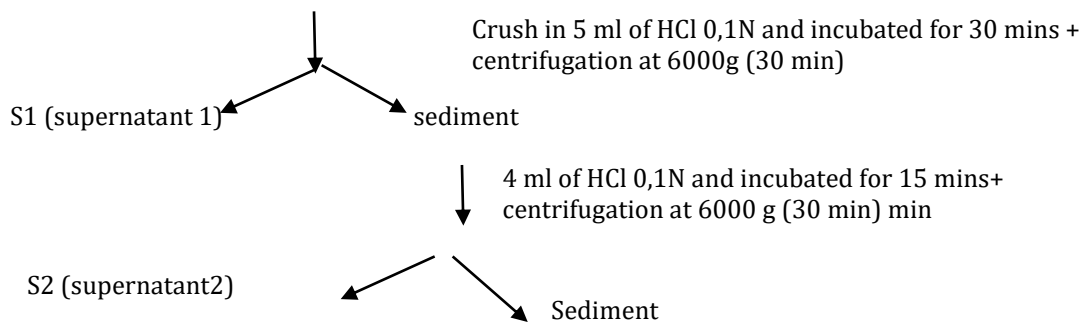
The biochemical test was carried out to determine the chemical compounds of resistance synthesized by each variety under the influence of the different treatments.

Two leaves per plot (six per combination) were taken from the field early in the morning, put in the bag containing ice and brought back to the laboratory to measure the quantity of phenolic compounds, the quantity of total protein and to determine the enzymatic activity of polyphenol oxidase (PPO), peroxidase (POX) and Glucanase. Each assay was repeated three times.

Extraction and determination of phenolic compounds

Extraction of total phenolic compounds was carried out according to Boudjeko *et al.* (2007) with slight modification. The protocol of extraction is described below.

The extraction steps are summarized as shown below;



$S_1 + S_2$ = crude extract of the total phenolic compounds According to the protocol described by Marigo (1973), the number of phenolic compounds was determined using the Folin-ciocalteu reagent. The Folin-Ciocalteu

reagent is reduced in an alkaline medium and under heat during the oxidation of the phenolic compounds into a blue mixture of tungsten oxide (W8O23) molybdenum oxide (Mo8O23), whose absorbance at 760 nm is

proportional to the quantity of phenolic compounds present in the extract. The content of phenolic compounds was expressed in $\mu\text{g/g}$ of fresh material (FM) regarding the curve established with gallic acid (0.1 mg/mL).

Extraction and determination of proteins

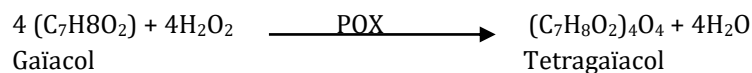
Protein extraction was performed according to the modified protocol of Pirovani et al. (2008). 1 g of leaves was crushed at 4°C in 5 ml of tris-maleate buffer (10 mM, pH 7.2). The crushed material was homogenized for 2 min at 4°C and centrifuged (Beckmann-Coulter microfuge 20 R) for 25 min at 10,000g at 4°C. The supernatant was collected and stored while the residue was resuspended in 3 mL buffer and centrifuged again at 20000xg for 20 min at 4°C. The resulting mixture of supernatant constitutes the protein extract which was stored at -20°C for further use.

The determination of total proteins in the extracts

obtained was carried out according to the method of Bradford (1976). In each test tube, 10 μL of extract, 490 μL of distilled water and 500 μL of Bradford's reagent were mixed and incubated in the dark for 15 min. The optical density was measured at 595 nm using the spectrophotometer (Shimadzu UV-1605, UV-visible). The concentrations of proteins present were expressed in μg as BSA equivalence/g fresh matter (FM) with reference to the calibration curve performed under the same conditions as the samples using BSA (Bovine Serum Albumin) 0.1 mg/mL.

Assessment of peroxidasic activity (POX) of proteins

The determination of peroxidase activity in the protein extract was performed according to the modified protocol of Hadrami and Baaziz (1995). It is based on the oxidation of guaiacol by peroxidases into tetra guaiacol in the presence of H_2O_2 according to the following reaction:



The 925 μL of Tris-Maleate buffer (10 mM, pH 7.2 and containing 1 g of guaiacol), 25 μL of protein extract and 50 μL of 10% H_2O_2 were mixed and incubated at room temperature for 3 min. The activity was measured following the formation of tétragaïacol at 470 nm using the spectrophotometer (Shimadzu UV-1605, UV-visible). Enzyme activity was expressed per gram of fresh material.

Assessment of polyphenoloxidase (PPO) enzyme activity

The activity of polyphenol oxidases (PPO) was evaluated in the protein extract according to Van Kammen and Brouwer (1964) using catechin as a substrate. 500 μL of 0.66 M phosphate buffer and pH 7, 150 μL of 10 mM catechin, 35 μL of protein extract were mixed and incubated at 25°C for 30 s. Absorbance at 330 nm was measured after five minutes using the spectrophotometer (Shimadzu UV-1605, UV-visible). Enzyme activity was expressed as Abs330/min/g of fresh material.

Assessment of enzymatic activity of glucanases

The beta-1,3-glucanase activity was evaluated in the protein extract according to the modified protocol of Leelasuphakul *et al.* (2006) using laminarin 0.0025% (g/mL), which is a polymer of β -1,3-glucan. The test tubes

were introduced successively, 90 μL of sodium acetate buffer pH 4, 0.1 M containing laminarin (25mg/L), 10 μL of protein extracts. The mixture was incubated for 10 min at 40°C in a water bath, then 200 μL of 2 M HCl was added to stop the reaction. The optical density was measured at 540 nm. The amount of sugar released was calculated from the standard glucose curve [OD = f (glucose concentration)]. The enzyme activity expressed in μmole of glucose released /min /g of fresh material.

Assessment of treatments on stalk length and yield

In each plot, ten plants were randomly selected to assess the effect of treatments on maize growth (plant height). The evaluation was done 21 and 50 DAS. A tape measure was used for these different measurements. The husks were removed at harvest, and the corn without husks per plot was subjected to the yield assessment. The weight of the cob without spathes of each plot was evaluated. Forty-five cobs per plot were measured.

Statistical analysis

The data were analyzed using SPSS 20.0 software. Duncan test at 5% was used to separate the means.

RESULTS

Efficacy of AENS and Mancozeb 80 WP on Incidence

and severity of PBS

The incidence of Physoderma Brown Spot increased gradually throughout the season. A significant difference ($P < 0.05$) was obtained between the different treatments. The highest incidence was obtained in the control treatment 1.4% 28 DAS and 52.39% 70 DAS. On the other hand, it was reduced in the AENS and Mancozeb treatments. The lowest incidence was recorded with the AENS treatment: 0.9% 28 DAS and 40.3% 70 DAS. Incidence remained significantly lower with neem compared to Mancozeb (Table 1).

Similarly, the highest severity was obtained with the control treatment, respectively 16.66% and 50.76% 28 DAS and 70 DAS. The lowest severities were recorded with the AENS and Mancozeb treatments. However, no significant difference was obtained in 70 days when the severities were 40.7% and 41.6% for AENS and Mz (Table 2). The rate of reduction of incidence and severity of PBS, compared to the control, at the last week of evaluation (70 DAS), was 23.1 and 19.9 %, respectively, with the AENS and 9.3 and 18.1 % the Mz.

Area Under Disease Incidence Progress Curve (AUIPC) of PBS

Throughout the campaign, the AUIPC of Physoderma Brown Spot recorded on variety CMS9015 in the control treatment remained significantly high ($P < 0.05$) than those of plots treated with Mancozeb 80 WP and AENS. However, the AUIPC of Mancozeb remained lower than that of AENS during the trial (Figure 1 A).

The evolution of the AUIPC curve of PBS at CMS8704 showed that leaf brown spot evolved significantly ($P < 0.05$) slowly in the AENS treatment but was higher in the control and mancozeb treatments (Figure 1 B).

Overall, the evolution of the AUIPC curves of both varieties (CMS9015 and CMS8704) showed a significant reduction of PBS in the Mz and AENS treated plots compared to the control. However, the disease was more reduced in the variety CMS8704 with these treatments than the variety CMS9015.

Area Under Disease Severity Progress Curve (AUSiPC) of PBS

The AUSiPC of PBS was higher in the control plots compared to plots treated with AENS and Mz during the trial in CMS 8704. However, at 63 DAS, no significant difference ($P > 0.05$) was obtained between

AENS and Mz (Figure 2A).

Similarly, for CMS 9015, AUSiPC was significantly ($P < 0.05$) higher in the control plot and lower in the Mancozeb and AENS plots. The effect of AENS on PBS severity was higher than that of Mz (Figure 2 B).

Globally, Area under disease severity curve was lower with CMS8704 than with CMS9015.

Efficacy of AENS and Mancozeb 80 WP on Incidence and severity of PSR

The incidence and severity of stalk rot evolved significantly ($P < 0.05$) during the season. However, the disease was higher in the control plot than in the plots that received Mancozeb 80 WP (Mz) and aqueous neem extract (AENS).

The incidence of PSR was higher in the control plot where it increased from 6.2%, 42DAS to 37.7%, 70 DAS. On the other hand, it remained low in the plots treated with Mz and AENS with respectively 4.7 and 2.6% 42 DAS against 25.3 and 13.5%, 70 DAS. AENS was very effective in reducing the incidence of SSR throughout the campaign (Table 3).

The lowest severity was recorded with AENS treatment with 25%, 42DAS and 36%, 70 DAS. The highest value was obtained in the control treatment with 27.6%, 42 DAS and 42%, 70 DAS (Table 4).

AENS was more effective than Mz in reducing the incidence and severity of PSR. AENS reduced incidence and severity by 64.2 and 15.5 per cent, respectively, compared with 32.9 and 6.1 for Mz.

Area Under Disease Incidence Progress Curve (AUIPC) of PSR

The evolution of the AUIPC curve of PBS at CMS8704 showed that Physoderma Stalk Rot was significantly ($P < 0.05$) lower in the AENS treatment and higher in the control and mancozeb treatments (Figure 3 A).

AUIPC of Physoderma Stalk Rot (PSR) recorded in variety CMS9015 was significantly ($p < 0.05$) higher in the control treatment than in the other treatments (Mancozeb 80 WP and AENS). However, the AUIPC of Mancozeb remained higher than that of AENS during the trial (Figure 3 B). The evolution of the AUIPC curves of both varieties (CMS9015 and CMS8704) showed a significant reduction of PSR in the AENS treated plots compared to the control. However, the disease was more reduced in the variety CMS8704 with these treatments than the variety CMS9015.

Table 1: Mean PBS incidence on maize at different growth stages.

Variety	Days after sowing																				
	28DAS			35DAS			42DAS			49DAS			56DAS			63DAS			70DAS		
	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN
CMS9015	2.1	0.8	1.4	6.9	3.21	5.03	19.4	11.2	13.6	28.8	17.7	21.6	49.5	43.7	42.7	51.2	42.5	45.7	53.1	43.5	46.8
CMS8704	0.7	0.4	0.4	9.1	12.7	8.31	26.8	27.5	19.4	37.1	43	21.3	44.2	43.7	31.8	47.7	49.6	33.5	51.7	41.5	33.8
Mean	1.4 a	0.6 b	0.9 B	8 a	7.9 a	5.03b	23.1a	19.5b	16.5c	33.5a	30.4b	21.4c	46.8a	43.7b	37.2c	49.4a	46.0 b	39.5c	52.4a	47.5b	40.3c

Means within a growth stage bearing the same letters are not significantly different by Duncan test at 5% level of probability. C : control, Mz : mancozeb ; EN : aqueous extract of neem seed.

Table 2: Mean PBS severity on maize at different growth stages.

Variety	Days after sowing																				
	28DAS			35DAS			42DAS			49DAS			56DAS			63DAS			70DAS		
	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN
CMS9015	16.7	16.7	8.3	21.3	25.0	28.1	38.3	33.8	32.7	44.5	37.2	40.7	49.5	42.3	41.1	48.5	42.5	45.7	54.4	43.5	36.6
CMS8704	16.7	16.7	8.3	34.1	30.7	28.3	40.4	34.7	32.5	47.0	42.5	42.4	49.3	43.9	36.6	50.5	39.2	34.6	47.1	37.7	46.8
Mean	16.7a	16.7a	8.3 B	27.7 a	27.9 a	28.2b	39.1a	34.2b	32.6c	45.7a	39.9b	41.6b	49.4a	43.9b	38.3c	49.5a	40.8 b	40.2b	50.8a	41.6b	40.7b

Means within a growth stage bearing the same letters are not significantly different by Duncan test at 5% level of probability. C : control, Mz : mancozeb ; EN : aqueous extract of neem seed.

Table 3: Mean PSR incidence on maize at different growth stages.

Variety	Days after sowing														
	42DAS			49DAS			56DAS			63DAS			70DAS		
	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN
CMS9015	6.8	4.8	2.9	25.1	10	8.3	28.1	13.7	10.4	34.5	16.5	12.3	33.7	12.7	12.6
CMS8704	5.7	4.6	2.4	22.6	11.9	9.3	25.4	13.7	10.1	33.4	26.5	11.9	35	30.9	14.4
Mean	6.2a	4.7b	2.6c	23.8a	10.9b	8.8b	28.1a	13.7b	10.3c	33.8a	21.5b	12.1c	37.7a	25.3b	13.5c

Means within a growth stage bearing the same letters are not significantly different by Duncan test at 5% level of probability. C : control, Mz : mancozeb ; EN : aqueous extract of neem seed.

Table 4: Mean PSR severity on maize at different growth stages.

Variety	Days after sowing														
	42DAS			49DAS			56DAS			63DAS			70DAS		
	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN	C	Mz	EN
CMS9015	27.8	28.2	25	33.8	30.1	29.8	39.3	33.8	32.2	40.5	39.1	35.2	41.6	39.3	35.5
CMS8704	28.1	27.5	25	39.7	28.8	25	39.9	38.1	33.6	41.0	39.9	37.0	42.6	40.7	36.5
Mean	27.6a	27.9a	25b	36.8a	29.5c	27.4b	39.6a	36b	32.9c	40.8a	39.4a	36.1b	42.0a	40b	36c

Means within a growth stage bearing the same letters are not significantly different by Duncan test at 5% level of probability.

C: control, Mz: mancozeb, EN: aqueous extract of neem seed.

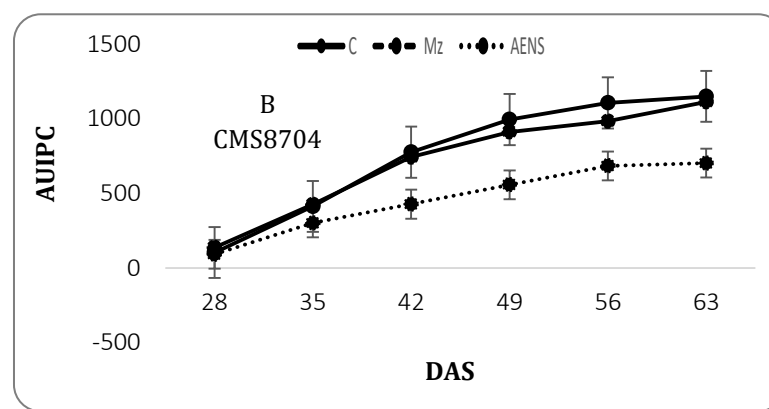
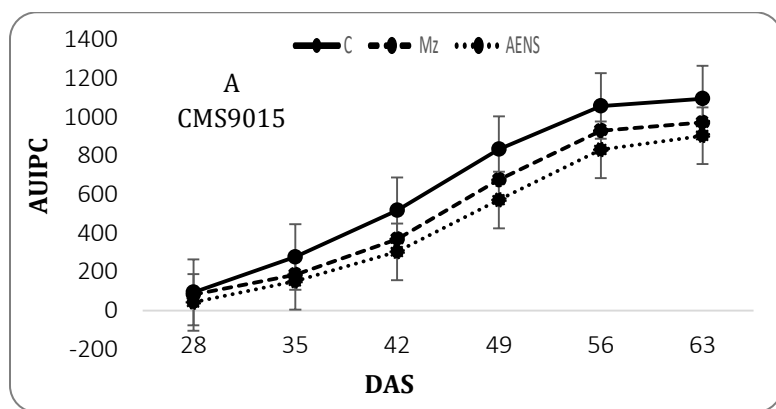


Figure 1 : AUIPC (Area Under the Disease Incidence Progress Curve) of Physoderma Brown Spot on maize leaves treated with Mancozeb 80 WP and AENS for 70 days on CMS8704 and CMS9015 varieties.

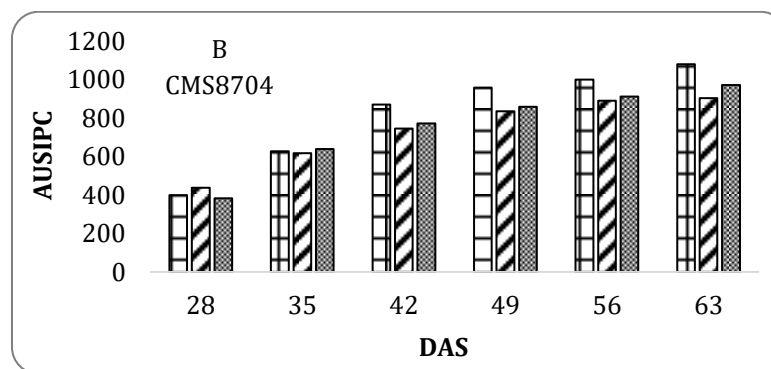
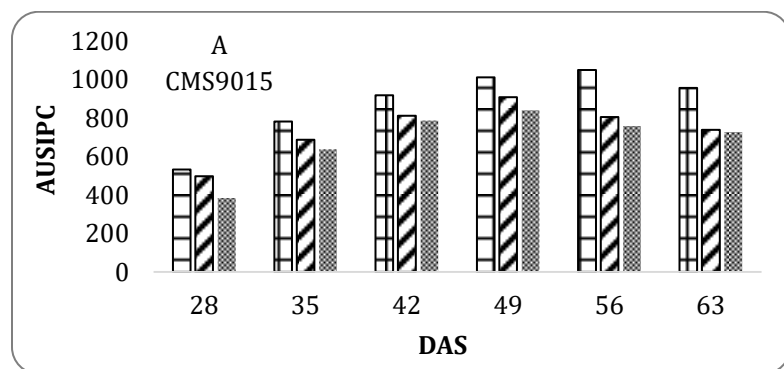


Figure 2 : AUSiPC (Area Under the Disease Severity Progress Curve) of Physoderma Brown Spot on maize leaves treated with Mancozeb 80 WP and AENS for 70 days on CMS8704 and CMS9015 varieties.

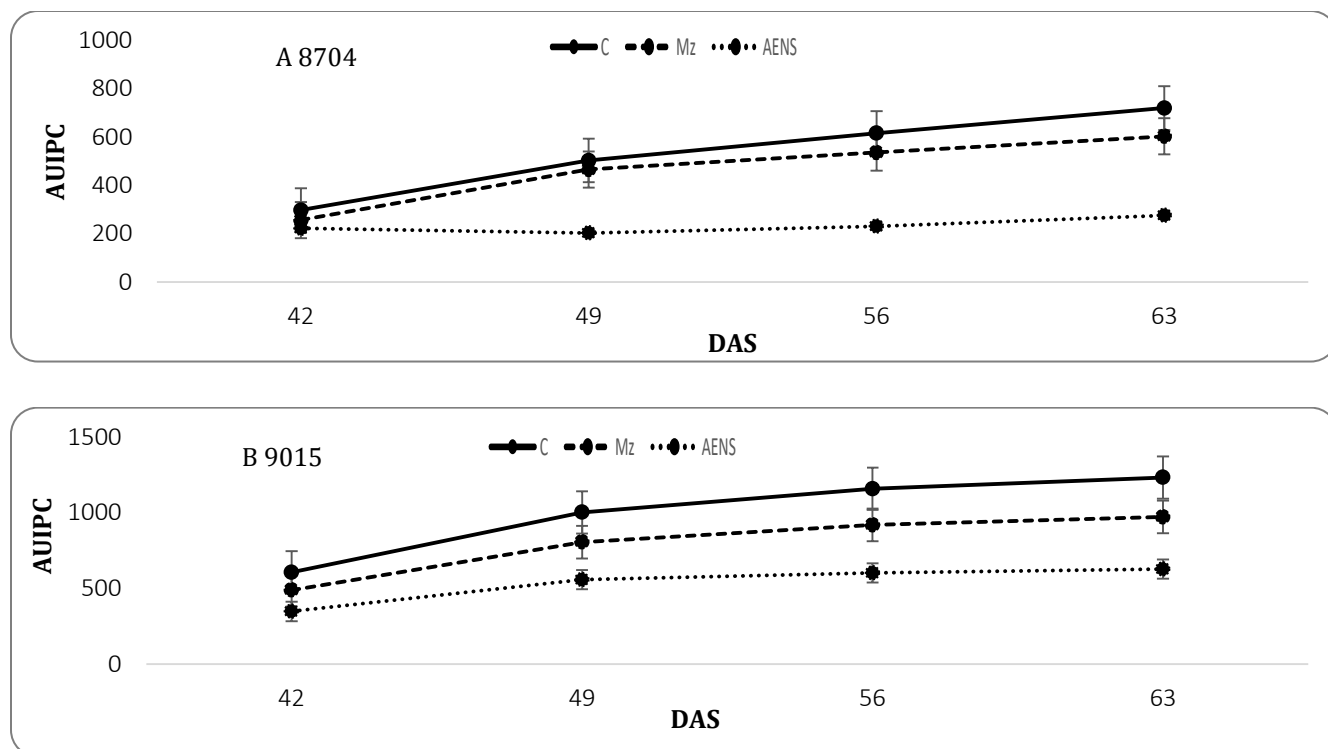


Figure 3 : AUIPC (Area Under the Disease Incidence Progress Curve) of Physoderma Stalk Rot on maize stalk treated with Mancozeb 80 WP and AENS for 70 days on CMS8704 and CMS9015 varieties.

Area Under Disease Severity Progress Curve (AUSiPC) of PSR

The AUSiPC of PSR was higher in the control plots compared to plots treated with AENS and Mz during the trial in CMS 8704 (Figure 4 A). However, a highly significant difference ($P < 0.001$) was obtained between AENS and Mz (Figure 4A).

Similarly, AUSiPC was significantly ($P < 0.05$) higher in control Mz plots and lower in the AENS plot. The effect of AENS on PBS severity was higher than that of Mz. No significant difference was obtained between control and Mz treatments (Figure 4 B).

Effect of treatment on the induction of resistance of both varieties

Effect of AENS and Mz on the enzymatic activity of PPO, POX and Glucanase

In CMS 8704, the polyphenol oxidase activity was higher in the AENS treatment (4.22 ± 1.02) and lower in the control treatment (0.89 ± 0.09), with a significant difference ($P = 0.0001$) between the three treatments. CMS9015 recorded a value of 0.793 ± 0.01 with the AENS and 0.57 ± 0.03 with the control.

CMS8704 showed higher polyphenol oxidase activity compared to CMS9015 (Table 5).

A highly significant difference ($P = 0.0001$) was obtained between the different peroxidase (POX) activity treatments. POX activity was higher in plots treated with AENS, followed by Mz and lower in untreated plots in both varieties. In variety CMS8704, 1.55 ± 0.04 , 1.32 ± 0.04 and 1.14 ± 0.01 $\Delta A_{470}/\text{min/g FM}$ were recorded with AENS, Mz, and control, respectively. In contrast, in CMS9015 the lowest activity (0.24 ± 0.01) was recorded in control and the highest (0.79 ± 0.01) with AENS. These results show that CMS8704 has a higher POX activity than CMS9015 (Table 5).

The lowest glucanase activity values were obtained in the control treatment (1.15 ± 0.01 $\mu\text{mole}/\text{min/g MF}$) with CMS8704 and (0.34 ± 0.01 $\mu\text{mole}/\text{min/g FM}$) with CMS9015. In both varieties, glucanase activity was significantly ($P < 0.05$) higher with AENS followed by Mz. The values recorded were 1.15 ± 0.01 and 0.86 ± 0.01 $\mu\text{mole}/\text{min/g FM}$ with CMS8704 and 0.93 ± 0.01 and 0.59 ± 0.01 $\mu\text{mole}/\text{min/g FM}$ with CMS9015, respectively. The activity was more stimulated by AENS in the variety (CMS8704) (Table 5).

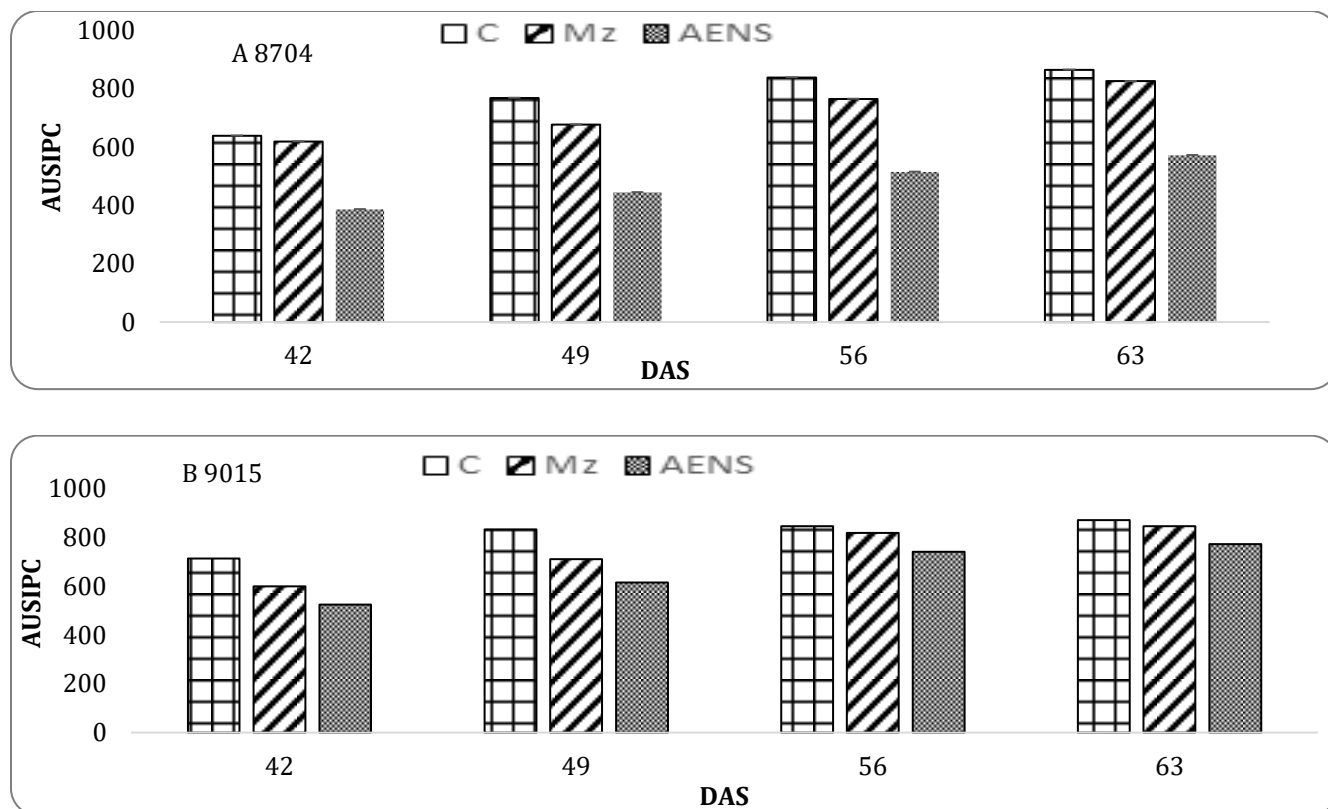


Figure 4 : AUSiPC (Area Under the Disease Severity Progress Curve) of Physoderma Stalk Rot on maize stalk treated with Mancozeb 80 WP and AENS for 70 DAS on CMS8704 and CMS9015 varieties.

Table 5: Enzyme activity measurements in both varieties.

Treatments	PPO activity ($\Delta A470/\text{min/g/FM}$)		POX activity ($\Delta A470/\text{min/g/FM}$)		Glucanase activity ($\mu\text{mole}/\text{min/g/FM}$)	
	CMS8704	CMS9015	CMS8704	CMS9015	CMS8704	CMS9015
Control	0.89 \pm 0.09a	0.31 \pm 0.03a	1.14 \pm 0.01a	0.24 \pm 0.01a	0.66 \pm 0.01a	0.34 \pm 0.01a
Mz	2.65 \pm 0.83b	0.57 \pm 0.03b	1.32 \pm 0.02b	0.40 \pm 0.01b	0.86 \pm 0.01b	0.59 \pm 0.01b
AENS	4.22 \pm 1.02c	0.79 \pm 0.01c	1.55 \pm 0.04c	0.58 \pm 0.01c	1.15 \pm 0.01c	0.93 \pm 0.01c

Values followed by the same letter on each variety show no significant difference according to Duncan's test at the 5% threshold.

Effect of AENS and Mz on the rate of phenolic compounds and total proteins of varieties

The contents of phenolic compounds are between 1.80 and 8.81 mg/g of FM and 4.7 and 6.5 mg/g of FM, respectively, for the variety CMS9015 and the variety CMS8704. A significant difference ($P=0.0001$) was noted between AENS and Mz in both varieties. However, no significant difference ($P=0.91$) was obtained between control and Mz with CMS8704. The production of phenolic compounds was higher in CMS9015 with AENS. The level of phenolic compounds was higher in the control of variety CMS8704 than in CMS9015 (Figure 5).

The highest amount of protein was obtained in the AENS treatment of the CMS8704 variety (25.13 ± 0.55 mg/g MF) and the lowest in the control treatment of the CMS9015 variety (1.39 ± 0.4 mg/g MF). Overall, protein levels were higher in CMS8704, and production was stimulated by AENS in both varieties. Statistical analysis showed no significant difference ($P=0.277$) between control and Mz in variety CMS8704 (Figure 5).

DISCUSSION

The incidence of the disease increases from 28 DAS. This reflects the onset of symptoms at around 21 DAS

(vegetative stage). The first rains in early June (80 mm) caused the germination and spread of zoospores released by the sporangia. These results corroborate those of Robertson *et al.* (2015), who found that *Physoderma maydis* can infect any plant part, but leaves are the most vulnerable. Moreover, in the control plants, the increase in incidence and severity was in accordance with the growing stage. The site's

history reveals that only organic fertilizer (cow dung) has been used continuously for the past ten years. This would justify the presence of the disease all over the field as *Physoderma maydis* lives in infected soils, the debris of the corn infected for several years. In addition, many authors have shown the presence of inoculum in animal faeces (Mueller and Wise, 2016; Tisdale, 1919).

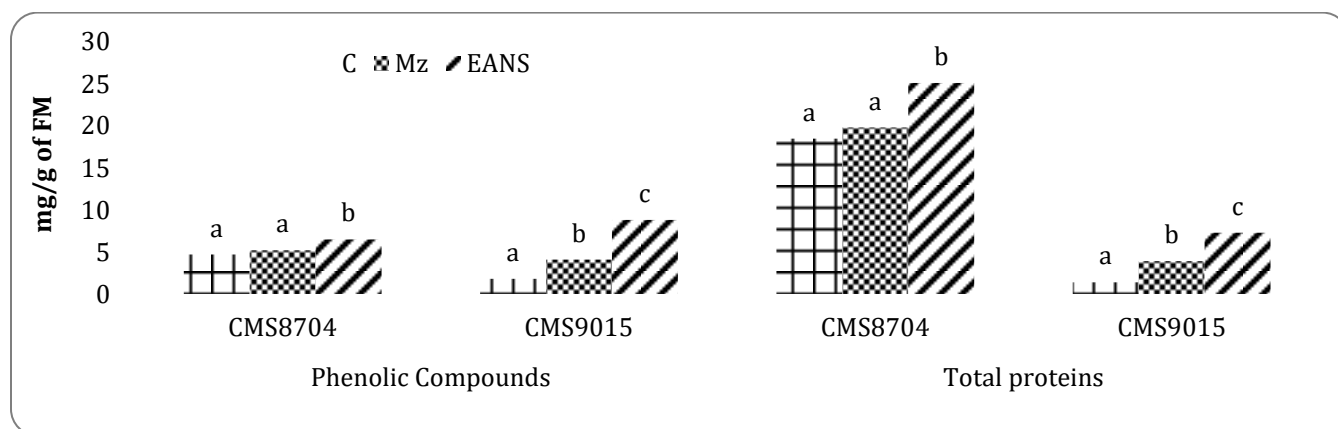


Figure 5: Production of phenolic compounds and total protein in both varieties.

Results obtained after application of Mancozeb 80 WP against *Physoderma maydis* showed that the incidence and severity of PBS and PSR in maize in the field was significantly reduced on both varieties. This reflects the efficacy of the active ingredient of Mz, which is a contact fungicide of the dithiocarbamate family. No studies are available on the efficacy of mancozeb 80 WP on *P. maydis*. Nevertheless, several works have shown the efficacy of Mancozeb 80 WP on other species of fungi in the field. Tonon *et al.* (2018) showed the reduced incidence and severity of cashew tree anthracnose caused by *Colletotrichum gloeosporioides* in the field. Randriantsalama *et al.* (2014) also showed that Mancozeb applied at 3kg/ha significantly reduced the incidence of late blight caused by *P. infestans* on potatoes. Duamkhanmanee (2008) and Chand *et al.* (2013) showed that the uses of Mancozeb 80 WP effectively reduced anthracnose due to *Colletotrichum lindemuthianum* in bean fields and mango anthracnose infections due to *Colletotrichum gloeosporioides*. Sikirou *et al.* (2012) had shown that Mancozeb was very effective in reducing the development of cercosporiosis caused by *Cercospora beticola* on lettuce. Moreira *et al.* (2019a) and Velho *et al.* (2015) have also shown the sensitivity of Dithiocarbamates fungicide against *C.*

acuminata and *C. boninense* in Brazil.

Moreover, AUIPC of PBS and PBR when treated with Mz were lower compared to the control. This result corroborates that of Ogoshi (2020), who have recently demonstrated that the fungicides mancozeb or its association with trifloxystrobin showed the lowest AUDIPC in relation to control against *Glomerella* leaf spot in Brazil. Tonon *et al.* (2018) also obtained a reduction in AUDIPC of anthracnose caused by *C. gloeosporioides* when treated with Mancozeb 80 WP. No study previously demonstrated the efficiency of neem extract against *P. maydis*. However, the results obtained after application of EANS against *Physoderma maydis* showed that the incidence and severity of PBS and PSR of maize in the field were significantly reduced on both varieties. This indicates that the active ingredient azadirachtin from neem was effective against *P. maydis*. However, AENS gave a more satisfactory result than Mz, confirming the efficacy of natural products based on plant extracts. One advantage of plant extract is that they contain more than one antifungal compounds (Shuping, 2016). Plant neem extracts have already shown efficiency against mainly fungi diseases. Pohe and Agneroh (2013) demonstrated the efficacy of Neem oil aqueous extract in reducing *Phytophthora* spp

attacks on cocoa pods compared to Ridomil 66 WP. Similarly, (Shu *et al.*, 2015) showed the efficacy of Neem extracts on diseases and pests in cherry production. In Canada, through its active ingredient azadirachtin, Neem has been extensively tested in horticulture and forestry with positive results on fungi (Dai, 1999). Wang *et al.* (2010) demonstrated that Neem extract at 200g/L dose effectively reduced the attack of fruits by certain pathogens. Mboussi *et al.* (2016) showed that aqueous neem seed extract reduced the incidence and severity of brown rot of cocoa pods in the field.

Numerous studies have shown that plant extracts, or their derived compounds have been used to control fungal diseases in the field instead of chemical compounds (Shabana *et al.*, 2017; Shuping, 2016). The advantage of plants is the non-toxicity to humans and the environment and the non-development of fungal resistance to these extracts. In addition, plants treated with plant extracts are organic and more expensive. However, FRAC (2020) showed that mancozeb caused less risk of resistance development, but some studies have already reported several pathogens (*Colletotrichum* species) resistant to this multi-site fungicide (Moreira *et al.*, 2019b).

The results of the biochemical analyses obtained show that AENS stimulated the activity of enzymes (PPO, POX and Glucanase) in the corn leaves on the one hand, and increased the amount of phenolic compounds and total proteins in the corn leaves on the other hand. This result could explain the low attack rate and a decrease in the severity of PBS and PSR observed in the AENS treated plots. These enzymes are involved in the defence mechanisms of some pathogens (Pierre *et al.*, 2015). Surendra *et al.* (2012) have demonstrated that extract of neem seed enhanced PPO and POX activity in mustard after application and induce resistance against *Alternaria brassicae*. Bharathi *et al.* (2004) recorded an increase in β -1, 3 glucanase, PPO, POX in plants treated with neem extract for the management of fruit rot infection in chillies.

Several other authors have already demonstrated the interaction of PPO and POX in plant disease resistance (Ondobo *et al.*, 2017). Masoko and Eloff (2005) have demonstrated the effect of terminalia species extract to induce resistance by enhancing PPO activity of pea against *Erysiphe polygoni*. Through its proteolytic activity, PPO is involved in the defense against several pathogens by accelerating cell death and pathogen

progression in the plant. Phenolic compounds can accumulate and actively participate in defence of plants against attackers (Bektas and Eulgem, 2015). Moreover, the results obtained showed an increase in the synthesis of phenols and soluble proteins in the plants after the treatment. This corroborates the results obtained by Goel and Paul (2015), who demonstrated the effectiveness of Neem aqueous extract in the induction of acquired systemic resistance (SAR) in tomato against *Pseudomonas syringae*. Paul and Sharma (2002) have shown that neem aqueous extract of leaves induced resistance against soil-borne pathogenic fungal (*Dreschlera graminea*) through the exhibition of rapid accumulation of phenolic compounds in host plant. Vera *et al.* (2011) and Craigie (2010) have shown that laminarin plant extract elicitor was efficient to enhance the production of phenol and proteins against *Fusarium solani* and *Erwinia* sp. These phenolic compounds make it difficult for pathogens to penetrate by reinforcing the rigidity of the pectocellulosic wall.

Mz has also been shown to be effective in the stimulation of resistance in maize varieties. Certain chemicals or their derivatives are known for their eliciting action on cultivated plants. William Norbert *et al.* (2018) showed that the activity of glucanase (PR2), polyphenol oxidase and peroxidase were very high in maize and rice plants treated with 24-epibrassinolide (RBE) compared to the control. In addition, Rodriguez-Salus *et al.* (2016) have showed the effect of the Synthetic Elicitor 2-(5-Bromo-2-Hydroxy-Phenyl)-Thiazolidine-4-Carboxylic Acid on reducing root growth beside inducing defense of *Pseudomonas syringae* pv tomato (Pst) strain DC3000 in *Arabidopsis*.

The yellow variety (CMS8704) was more resistant to *Physoderma maydis*. This result could be explained by the high rate of secondary metabolism and enzyme activity in this variety. Ngadze *et al.* (2012) reported that tolerant genotypes accumulate more biochemical substances such as phenols, POX and PPO.

Cob weight yield of each variety confirmed that AENS increased corn yield compared to Mancozeb and the control. Fungicides are often found to reduce disease but do not impact yield (Hanna *et al.*, 2008).

CONCLUSION

Mancozeb 80 WP and neem aqueous extract were effective against *P. maydis*. The incidence and severity of PBS were reduced by 23.1% and 19.9%, respectively, by

AENS and by 9.3 and 18.1% by Mz. SSP was reduced by 64.2 and 15.5 per cent by AENS and by 32.9 and 6.1 for Mz. AENS and Mz stimulated variety resistance by increasing enzyme activity and production of phenolic compounds in the varieties. Neem and Mancozeb 80 WP can be used in IPM against *P. maydis*.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author's Contribution

NGOH DOOH JP, AMBANG Z, DJILE Boubou, DJONGNANG G participated in the design of the study and data analysis. NGOH DOOH JP, DJONGNANG G DEURNAYE P, MBOU TPR, YAMAGUI R, NTATSINDA CD, participated in sampling and data collection. TENE TPM and TENYANG Noel, performed biochemistries analysis, NGOH DOOH JP drafted the manuscript. All authors read and approved the final manuscript.

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