



***In vitro*, *In vivo* and *In situ*, Effect of Mancozeb 80 WP on *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., Causative Agent of Anthracnose of Cashew (*Anacardium occidentale* L.) in Chad and Cameroon**

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NDJP and AZ designed the study, wrote the first draft and performed the statistical analysis of the manuscript. Author DP wrote the protocol. Authors DP, AM, MTPR and DG participated in sampling and data collection. Author DB managed the analyses of the study. Author BFU managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2021/v6i330161

Editor(s):

(1) Prof. John Yahya I. Elshimali, UCLA School of Medicine & Charles R. Drew University of Medicine and Science, USA.

Reviewers:

(1) Haidel Vargas Madriz, Universidad de Guadalajara, México.

(2) Ivana Mitrović, University of Novi Sad, Serbia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/65633>

Original Research Article

Received 01 January 2020

Accepted 03 March 2021

Published 15 March 2021

ABSTRACT

Aims: The aim of this work was to evaluate effect of Mancozeb 80 WP against *Colletotrichum gloeosporioides*, the agent responsible of anthracnose of cashew tree (*Anacardium occidentale* L.).

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Study Design: The experimental design was in completely randomized blocks with three replications.

Place and Duration of Study: *in vitro* and *in vivo* experiments were performed in Laboratory of Department of Biological Sciences, University of Maroua during six months. Field trial was carried out in Kelo, Chad during three months.

Methodology: Isolates were obtained from diseased organs (leaves and fruits) from Kélo in Chad and Maroua in Cameroon. The concentrations used in the laboratory were C₁ (5 mg/ml), C₂ (0.5 mg/ml), C₃ (0.05 mg/ml), C₄ (0.005 mg/ml), C₅ (0.0 mg/ml). Radial growth, sporulation, conidial germination and pathogenicity were used to characterize and evaluate the effect of Mancozeb on the isolates *in vitro*. The preventive test was performed on three-month-old plants previously treated with Mancozeb. The concentration of 5 g/l was applied to the field and the incidence and severity were used to calculate the AUIPC (Area Under Disease Incidence Progress Curve) and AUSiPC (Area Under Disease Severity Index Progress Curve) curves.

Results: Mancozeb reduced radial growth of all isolates at concentrations C₁ (5 mg/ml), C₂ (0.5 mg/ml) and C₃ (0.05 mg/ml). The percentages of inhibition ranged from 50 to 100%. Mancozeb 80 WP completely (100%) inhibited the germination of *C. gloeosporioides* conidia *in vitro*. Mancozeb has protected cashew plants *in vivo* at the concentration C₁ (5 mg/ml). AUIPC and AUSiPC were higher on control plants and lower on Mancozeb-treated plants.

Conclusion: Mancozeb 80 WP may be associated in integrated pest management strategy against anthracnose.

Keywords: *Anacardium occidentale* L.; *Colletotrichum gloeosporioides*; anthracnose; Mancozeb 80 WP; inhibition; AUIPC; AUSiPC; mancozeb; *Anacardium occidentale* L.; pathogen.

1. INTRODUCTION

Demographic studies have shown that the accelerated rate of population growth in the world and particular in Africa is the cause of hunger and malnutrition. About 800 million people with majority in developing countries are affected [1,2]. To try to solve this problem, many countries improve their agricultural yield in general and particularly, cultivation and marketing of the cashew tree (*Anacardium occidentale* L.). Cashew nut cultivation contributes to the socio-economic and ecological development of several countries in the world. It participates in the conservation of biodiversity and the reconstitution of degraded and impoverished crop lands [3,4]. The nut is used in several fields including food, cosmetics, medicine and automotive industry [5].

Global cashew production increased from 2,361,384 tons in 2002 to 4,152,315 tons in 2012 with approximately 7.5 million hectares of plantations in 32 countries. This makes cashew (*Anacardium occidentale* L.) one of the leading crops with a currency of US\$1.12 billion [2].

Cashew tree cultivation is more developed in West Africa with Ivoire Coast, Benin, Nigeria than in Central Africa. Since 2015, Ivory Coast has become the world's largest producer and exporter of cashew nuts with more than 700,000 tons [6]. In Africa, cashew production is

nowadays a booming cash crop and represents a great opportunity through the export of its nuts [7].

In Chad, cashew production is 11.76 tons per hectare. Numerous projects have launched cashew tree production in the localities of Kélo, Moundou, Doba, Sarh. In Cameroon, the cashew tree is currently popularized in the North of Country (Adamaoua, North and Far North), in the East and in the Center through major projects coordinated by the Ministry of Scientific Research of Development and Innovation and IRAD [4] through the free distribution of nearly 300,000 cashew tree seedlings to about thirty farmers' organizations (cooperatives and joint initiative groups) and about fifty individuals [8,9].

Despite its socio-economic impact, cashew tree production is threatened with pests (*Zographus regalis* Brown, *Helopeltis anacardii* Miller, *Analeptes trifasciata* F. *Apate terebrans* Pallas) and diseases (Anthracnose, Red Rust, Bacteriosis, Black Rust, Powdery mildew, Alternaria, virosis, dieback, bacteriosis). Among these diseases, the most important is anthracnose, which often causes yield losses of about 70-100% [10-13]. These large yield losses represent significant income losses for cashew producers [14].

The agent responsible of anthracnose is *C. gloeosporioides*, a highly fungus that attacks a

variety of crops like sorghum, mango, papaya, banana, olive and guava [15,16]. The fungus infects leaves, flowers and fruits and thus reduces orchard yields and fruit quality [17-19]. This fungus causes a reduction in plant photosynthetic surface area because of tissue necrosis and defoliation of leaves.

Chemical control is the most effective global method against cashew nut anthracnose [17,20-22]. Mancozeb 80 WP is a broad-spectrum, preventive contact fungicide. It is used to protect many fruits and vegetables and field crops against a wide range of fungal diseases such as mildew, anthracnose, scab and rust [17]. It is a multi-site inhibitor, toxic to fungal cell membranes. It contains mancozeb 80% (active ingredients), zinc and manganese, essential trace elements involved in plant metabolism.

However, in Chad and Cameroon, despite the high incidence of anthracnose in all orchards [23], no protective measures are undertaken by farmers. Furthermore, no studies have been conducted on the anthracnose pathogen in the two countries. Thus, the aim of this study is to evaluate the effect of mancozeb 80 WP against *Colletotrichum gloeosporioides*, the agent responsible for cashew nut anthracnose *in vitro*, *in vivo* and *in situ*.

2. MATERIALS AND METHODS

2.1 Plant Material

The study was carried out in Kélo, Chad (9° 18' N and 15° 48' E) and in Maroua, Cameroon (10° 35' N, and 14° 19' E). The rainfall in Kelo (Chad) and Maroua (Cameroon), was highest during July and September months. Precipitation recorded were respectively 318.5 mm and 270.5 mm in Kelo. In Maroua, it was 218 mm and 415 mm respectively. During the rainy season 831.2 mm were recorded in Kelo and 989 mm in Maroua (Table 1).

2.2 Sampling of Diseased Organs

Sampling was carried out on trees with leaves and fruits showing symptoms of anthracnose. Samples were randomly collected in 5 trees per orchards and placed in plastic bags containing water-soaked cotton. Each bag contained the location (country and city), the date of collation and the geographical coordinates of each locality. Isolates were named according to the location,

foot or organ attacked (ICK = Isolate of Chad Kélo and ICM = Isolate of Cameroun Maroua). They were then transported to the laboratory of the Faculty of Biological Sciences in Maroua for processing.

2.3 Obtention of Isolates and Identification of the Pathogen

To obtain the isolates, two culture media were used. The PDA medium (Potato Dextrose Agar) was prepared with 200 g potatoes, 20 g glucose and 15 agar. This medium was used for both isolation and purification of the isolates. The WA medium (Water Agar) was prepared using 15 g of Agar in 1 liter of hot water. This media were used for isolation only.

The leaves and fruits of the cashew tree showing symptoms were washed with tap water. These leaves were cut off towards the end of the symptom growing area. They were then disinfected with 70°C alcohol and then with 1% Sodium Hypochlorite for three minutes and rinsed three times with water sterile distilled. Finally, the fragments were dried on previously sterile filter paper [18].

The inoculation was performed aseptically. The previously disinfected fragments were introduced in the Petri dishes containing WA and PDA media at a rate of 4 fragments per dish. The Petri dishes were then hermetically sealed with paraffin and incubated at room temperature for 7 days under a 12/12 photoperiod.

Isolates were purified by removing uncontaminated mycelial discs from the peripheral growth zone of the colonies. These mycelial discs were carefully placed in Petri dishes containing a new PDA medium. This operation was repeated till obtention of pure culture of *Colletotrichum gloeosporioides*.

The identification of the isolates obtained has been made on the basis of macroscopic and microscopic aspects using several identification keys[12,24].

2.4 Characterization of the Different Isolates

Three parameters were used to characterize the different isolates obtained, radial growth, sporulation and pathogenicity.

Table 1. Rainfall recorded during the experiment

Week	Months June		July				August				September			October		
	W ₁	W ₂	W ₃	W ₄	W ₅	W ₆	W ₇	W ₈	W ₉	W ₁₀	W ₁₁	W ₁₂	W ₁₄	W ₁₅	W ₁₆	W ₁₇
PK*	49.2	14.2	75.2	77.3	0	166	0	18.7	20.5	66.6	101.7	65.8	0	103	73	-
PM*	80	70	0	80	78	60	50	30	40	70	175	135	80	25	5	3

*PK Precipitation (mm) in Kélo ; PK precipitation (mm) in Maroua; - not recorded

The plant material used consisted of leaves and fruits of cashew trees showing symptoms of the disease from different orchards in Kélo (Loukti, Djingreng, Coton Chad, Kolon Kassouda) and Maroua (Meskine, Djarengol Kodeck, Ourotchédé, Palar) and cashew tree 3 months old purchased in Maroua markets

2.4.1 Measurement of radial growth

Mycelial growth was measured by measuring two perpendicular diameters of the Petri dishes. Using a handle, fragments (mycelial disc) of 0.8 cm in diameter were obtained. They were placed in the center of each Petri dish, one fragment per dish, using a punch. For each isolate, three replicates were made. Colony diameters were measured daily from 48 hours until one of the colonies had filled the petri dish. Mycelial growth was calculated according to the formula of [25].

$$D = \frac{d1 + d2}{2} - d_0 \quad \text{Where: } D =$$

diameter of growth ; d_0 = diameter of mycelial disc; d_1 and d_2 = two perpendicular diameters.

2.4.2 Sporulation measurement of isolates

At the end of growth of each isolate, four mycelial discs of 0.8 cm diameter were taken from each Petri dish to count the spores. The mycelial discs were crushed in 1ml of sterile distilled water. The solution was sieved through a muslin cloth. After sieving, 0.1 ml of the suspension was withdrawn with an insulin syringe and calibrated with the Malassez cell (hematimeter) for conidial rate of each isolate. Each operation was repeated three times [26].

2.4.3 Pathogenicity test of isolates

The pathogenicity test was done on leaves. The leaves of plants about 3 months old were used to assess the pathogenicity of different isolates [21, 22].

2.4.3.1. Preparation, disinfection and inoculation of detached leaves

The leaves were washed with tap water, disinfected with ethanol (70 °C) for three minutes in a 1% sodium hypochlorite solution and then rinsed three times with sterile distilled water. The leaves were placed in a bac (box) containing filter paper soaked in sterile distilled water to maintain humidity. For each isolate, three leaves were prepared. The design was set up the day before (approximately 12 h). Three drops of the spore suspension (obtained from the pure cultures) of each isolate, calibrated to 3-4,105 spores/ml using the Malassez cell were deposited at three locations on each leaf. After inoculation, incubation was done under a 12/12 photoperiod [27].

2.4.3.2 Assessment of severity on leaves

Symptoms were read on the leaves 10 days after inoculation. The severity index was calculated based on a sensitivity scale [26]. Where, 0: absence of symptoms; 1: small points of penetration; 2: network points; 3: necrosis diameter between 0.5-1; 4: necrosis diameter between 1-2; 5: necrosis diameter > 2-3.

Koch's postulate which consists in reisolating the fungi from the leaves was performed ([13].

2.5 Evaluation of the Effect of Mancozeb 80 WP *in vitro*

The efficacy of Mancozeb 80 WP *in vitro* was evaluated on the growth and germination of conidia.

2.5.1 Preparation of different concentrations

The stock solution was obtained by diluting 1g of the fungicide in 100 ml of distilled water. From this stock solution, concentrations of 5; 0.5; 0.05 and 0.005 mg/ml were obtained. A sample of 30, 3, 0.3 and 0.03 ml of the stock solution was added to 30, 57, 59.7 and 59.97 ml of PDA medium, respectively, to give a total volume of 60 ml. Four concentrations have used C_1 (5 mg/ml), C_2 (0.5 mg/ml), C_3 (0.05 mg/ml), C_4 (0.005 mg/ml).

2.5.2 Effect of treatments on the growth of isolates

Using a punch, mycelial discs aged 6-7 days of culture were taken from plates of each of the isolates and aseptically deposited in the center of each Petri dish containing the different concentrations. A control treatment (C_0 (0.0 mg/ml)) has been prepared. Each treatment was repeated three times. The Petri dishes were incubated at room temperature. Radial growth was measured daily from 48 hours. The percentage of inhibition was calculated compared to the control.

2.5.3 Effect of mancozeb on conidial germination of the different isolates

PDA media supplemented with different concentrations of Mancozeb were poured onto the slides. Each treatment was repeated three times. A spore suspension of each isolate was calibrated to $4-5 \times 10^5$ and spread on slides containing supplemented PDA media and control

PDA media. The number of spores germinated was assessed by microscopic observation 48-72 hours later on three areas of each slide [28].

2.6 Evaluation of the Effect of Mancozeb on Cashew Tree Plants in Greenhouse

2.6.1 Preparation of the inoculum

The two most virulent isolates (ICK₄ and ICM₂) were selected. The 5-76 day old mycelium cultures were scraped and crushed in 5 ml of sterile distilled water. The resulting spore suspension was calibrated using the Malassez cell at 5.10⁵ spores/ml.

2.6.2 Treatment and inoculation of plants

Cashew tree of about 3 months old with six 6 or 7 leaves were used. These plants were cleaned with tap water, disinfected with alcohol at 70°C and then with 1% sodium hypochlorite and rinsed with sterile distilled water. For this preventive test, the plants were treated with Mancozeb 80 WP at concentrations C₁ (5 mg/ml) and C₄ (0.0005 mg/ml) 12 hours before inoculation. Control plants were treated with sterile distilled water. The spores were sprayed on the lower and upper surfaces of the leaves with a 10 ml syringe. Immediately, the plants were covered with white and transparent plastic to maintain humidity. At the end of the test, Koch's Postulate was applied [17]. Symptoms were observed after 14 days.

2.7 Assessment of efficacy of Mancozeb 80 WP in Field

2.7.1 Experimental Design

The experiment was carried out in the field at Kélo in Chad during the 2020 rainy growing season (July-October). The experimental design was in complete blocks (03 blocks) randomized with two treatments. The two treatments consisted of control plants and the plants treated with Mancozeb 80 WP at concentration of 5g/l. Each block contained 60 plants with 30 plants per plot. The field area was 3600 m².

For the treatment of plants, Mancozeb were diluted in 16 liters of water in a sprayer. The plants were sprayed with a hand sprayer at 14-day intervals after each treatment during two or three months. Control plots did not receive any treatment.

2.7.2 Assessment of disease incidence

The incidence of disease was assessed before the start of treatment and every two weeks after

treatment. It was calculated according to the following formula:

$$I (\%) = \frac{n \times 100}{N} \quad [29].. \text{ Where: } I =$$

Incidence of disease; n = Number of diseased plants; and N = Total number of plants assessed.

2.7.3 Assessment of disease severity

Disease severity on treated plants was estimated on a visual scale from 0 to 4 where: 0 = 0%; 1 = 25%; 2 = 50%; 3 = 75% and 4 = 100% following the north, south, east and west sides of the crown of each tree. It was determined by applying the following formula:

$$s = \frac{\sum ab}{N} \quad \text{Where: } S = \text{disease severity index in}$$

the site; Σ = sum of the products of the number of diseased plants; a = disease severity index on the tree; b = number of plants with the given index in % and N = total number of diseased plants observed in each plot.

2.7.4 Area under disease progress the curve (AUDPC)

To determine disease progression in the field after treatment, the **AUIPC** (Area Under Disease Incidence Progress Curve) and the **AUSiPC** (Area Under Disease Severity Index Progress Curve) were calculated [12,30,31]. The formula is as follows:

$$AUDPC = \sum_{i=1}^n (X_i + X_{i+1}) / 2(t)$$

X_i is the incidence of disease at the time i, X_{i+1} is disease incidence recorded at the time i + 1, n, the number of registration on the incidence, and t, days between the registration of X_i and X_{i+1}

2.8 Data Analysis

SPSS 20.0 software was used to perform statistical analyses. The comparison of means was done by Duncan's test at the 5% threshold.

3. RESULTS

3.1 Characteristics of Different Identified Isolates

A total of 10 isolates were selected, including 5 from Chad (ICK1, ICK2, ICK3, ICK4, ICK5) and 5 from Cameroon (ICM1, ICM2, ICM3, ICM4, ICM5). The strains obtained have a white color, rounded shape and cottony appearance. The

microscopic aspect revealed an important number of spores, short and long, without flagella.

3.1.1 Radial growth diameters of isolates

Statistical analysis revealed a significant difference ($P = 0.001$) between the growth diameters of the different isolates studied. Statistical analysis of the mean diameters observed on Day 6 showed three (3) homogeneous statistical groups. Isolate ICK4 had the smallest mean diameter of 6.20 cm. On the other hand, isolates ICK1 and ICM1 had the highest growth diameters with a mean value of 7.50 cm (Table 2).

3.1.2 Sporulation rate of isolates

Analysis of the results reveals that there is a significant difference ($P = 0.0001$) in the sporulation of the isolates studied (Table 2).

The sporulation of the different isolates varies from a lower level of sporulation (group a) to a high level of sporulation (group d). The ICM4 isolate showed a higher level of spore production (14.66×10^5 spores/ml). In contrast, isolate ICM1 had the lowest spore production level 1.33×10^5 spores/ml (Table 2).

3.1.3 Pathogenicity of isolates

The results showed a significant difference ($P = 0.0001$) among the different isolates studied (Table 1). Isolate ICM2 had a high level of aggressiveness (3.5%) followed by isolates ICK4, ICK2, ICM4 (2.5%). On the other hand, ICK1 showed a lower (0.5%) level of aggressiveness (Table 2).

3.2 Effect of Mancozeb on Radial Growth of Different Isolates

Statistical analysis of the results revealed significant differences ($P = 0.0001$) between the different concentrations. With the exception of the isolates ICM2 from Cameroon, ICK2 and ICK5 from Chad, the growth of all other isolates was totally inhibited (100%) at C1 concentration (Fig. 1). The percentages of inhibition ranged from 50 to 100% at C2 concentration (0.5 mg/ml). At C3 concentration (0.05 mg/ml), ICK1 and ICM1 isolates showed 5.9 cm against 3.17 cm for ICM3 (Fig. 1). The C4 concentration (0.005 mg/ml) slightly reduced the mycelial growth of the isolates. All concentrations reduced mycelial growth compared to the control. However, the

percentage of inhibition was function of the Mancozeb concentration (Fig. 1).

3.3 Effect of Mancozeb 80 WP on the Germination of Conidia of *C. gloeosporioides*

Analysis of the results of the *C. gloeosporioides* spore germination test showed a significant difference ($P = 0.0001$) between the control and the different concentrations of mancozeb (Table 3). Regardless of mancozeb concentration, inhibition of germination of *C. gloeosporioides* spores was complete (100%).

3.4 In vivo effects of Mancozeb on *Colletotrichum gloeosporioides*

The results showed a clear protection (absence of symptoms) of the plants treated at C₁ concentration (5 mg/ml) 14 days after inoculation (Fig. 2 a). However, symptoms of anthracnose were observed on plants treated (Fig. 2 b) at C₄ concentration (0.005 mg/ml) and much more on untreated plants (Fig. 2 c). The symptoms obtained from the test were identical to those at baseline and the pathogen reisolated was *Colletotrichum gloeosporioides*.

3.5 Inhibitory Action of Mancozeb on Anthracnose in the Field at Kélo

3.5.1 Effect of mancozeb 80 WP on incidence of disease

Statistical analysis showed a significant difference ($P = 0.002$) between the different treatments. The incidence of anthracnose, expressed as the area under the disease progress curve, on the plants before the treatments was 40%. After treatments with Mancozeb, it was reduced by 26, 16, 10 and 7% respectively at days 14, 28, 42 and 56. In contrast to plants not treated with Mancozeb, an increase in the incidence of anthracnose of 47, 58, 67 and 91% was obtained at 14, 28, 42 and 56 days respectively. The area under disease incidence curve (AUIPC) was higher for untreated plants and lower for plants treated with mancozeb (Fig. 3).

3.5.2 Effect of Mancozeb 80 WP on severity of disease

The severity of the anthracnose disease assessed before treatment on plants was 21%. It decreased significantly ($P = 0.001$) after

treatment by 18, 18, 16 and 14% respectively on days 14, 28, 42 and 56. On the other hand, an increase in severity was observed by 31, 46, 63 and 78% on no treated plants. The area under the curve (AUSiPC) was higher on no treated plants and lower on treated plants (Fig. 4).

4. DISCUSSION

The objective of this study was to contribute to the control of *C. gloeosporioides*, the agent responsible for cashew tree anthracnose. The different isolates obtained revealed the same macroscopic and microscopic aspects of the pathogen, *C. gloeosporioides*. This result shows that these isolates belong to the same fungus despite their origin.

High variability was obtained in the growth rate, sporulation and pathogenicity test of the different isolates. This diversity is due to the nature of the isolates and the influence of several factors such as light, temperature, humidity, time and place of collection and the state of the culture media. These results are in agreement with those of [32], who obtained nine groups at the same incubation period of isolates *C. gloeosporioides*. Mycelial growth plays an important role in the variability between strains of the same species [33].

Mancozeb was more effective in reducing mycelial growth in vitro of the fungus with the highest concentrations used compared to the other concentrations and the control. The efficacy of Mancozeb which belongs to the family would be due to its mode of action which disrupts microtubules whose activity is essential in nutrient transport and cell division. Our results are in agreement with those of [17,21]. Who showed a 100 % inhibition rate on the growth of *C. gloeosporioides* isolates following the application of Mancozeb 80 WP. In addition, [34,35,36]. reported that the fungicides Mancozeb 80 WP had an inhibitory effect on the radial growth of the fungus *Phytophthora infestans*, *Botrytis cinerea* and *C. gloeosporioides*.

The germination test showed 100% spore inhibition of *C. gloeosporioides* isolates in all different concentrations of Mancozeb. The total inhibition of germination would be explained by the action of Mancozeb on respiration and the cell division of the spores of the fungus. In fact, dithiocarbamates fungicides block thiol groups of fungi and disrupt their metabolism by inhibiting either nucleic acid synthesis or by inhibiting degradation of fatty acids or glucose oxidation.

Our results are in agreement with those of [21]. who showed a 100% inhibition rate on spore germination following the use of Mancozeb 80 WP. Moreover, [22]. obtained percentages of inhibition of germination of *C. gloeosporioides* spores of the order of 50% after application of Mancozeb *in vitro*. [37] had achieved more than 99% inhibition of *C. gloeosporioides* germination with mancoxyl, a contact and systemic fungicide containing mancozeb as one of the active ingredients.[36,38]. have shown the effectiveness of Mancozeb on spore germination of *C. gloeosporioides*. Multi-site fungicides are known by their action on respiration through the thiol (sulfur) function which associates with oxygen and thus limits respiration, which is an essential process of germination [37]. Studies have shown that Mancozeb also inhibits spore germination of many pathogenic fungi. [34] reported that Mancozeb 80 WP fungicides had an inhibitory effect on the germination of the fungus *Phytophthora infestans*. Other contact fungicides of the dithiocarbamate family, such as cymoxanil, have been shown to be effective in inhibiting germination of *C. gloeosporioides* spores [39,40].

The results of the *in vivo* effect of Mancozeb 80 WP, on *C. gloeosporioides* showed the efficacy of the fungicide as a function of concentration. Plants receiving the highest concentration of Mancozeb were protected compared to those receiving the low concentrations as well as control plants. The results confirm the preventive effect of Mancozeb 80 WP which is contact fungicide against fungal diseases in general and in particular, the pathogen responsible for cashew tree anthracnose. These results corroborate those of [22] who showed that the Propiconazole protected cashew tree plants against anthracnose *in vivo*. In addition, [41,42] showed the efficacy of Carbendazim in reducing mango anthracnose infections due to *C. gloeosporioides*.

The application of Mancozeb 80 WP on plants in the field significantly reduced the incidence and severity of plants treated with Mancozeb. This reduction confirms the effectiveness of the active ingredient, mancozeb, on the fungus *C. gloeosporioides*, an anthracnose pathogen. These results are in agreement with those of [21].who showed that field application of the fungicides Mancozeb and Chlorothalonil were more effective against anthracnose on cashew tree plants. Similarly, [43] reported that the fungicides Mancozeb and Chlorothalonil were

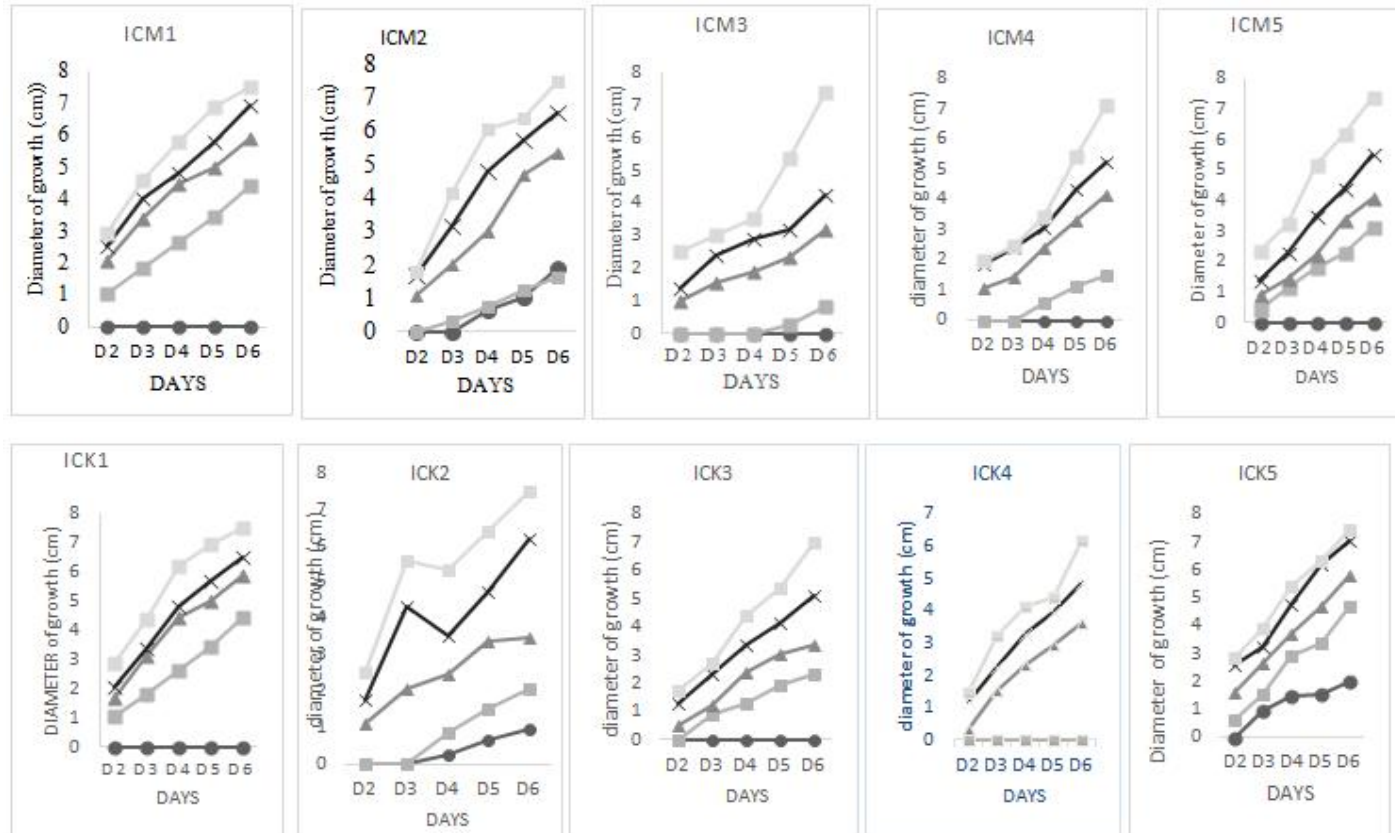


Fig. 1. Evolution of radial growth (in diameter) of 10 isolates of *C. gloeosporioides* in PDA medium supplemented with different concentration of Mancozeb 80WP



Table 2. Diameter, sporulation rate, pathogenicity of the different isolates

Isolate Code	Average diameters (cm)	Isolate codes	Number of spores ($\times 10^5$ /ml)	Isolate Code	Severity index (%)
ICK1	7.50 c	ICM4	14.66 d	ICM2	3.5 d
ICM1	7.50 c	ICM3	8.40 c	ICK4	2.5 cd
ICK2	7.48 c	ICK4	4.00 b	ICK2	2.5 cd
ICK5	7.47 c	ICM5	4.00 b	ICM4	2.5 cd
ICM2	7.45 bc	ICK5	3.36 ab	ICK3	2 bc
ICM3	7.40 bc	ICK3	3.33 ab	ICM5	2 bc
ICM5	7.36 bc	ICK2	3.00 ab	ICM3	2 bc
ICM4	7.13 bc	ICK1	2.33 ab	ICK5	1 ab
ICK3	7.10b	ICM2	2.33 ba	ICM1	1 ba
ICK4	6.20a	ICM1	1.33 a	ICK1	0.5 a

Table 3. Percentage inhibition of spore germination in different concentrations of Mancozeb

Isolates code	Treatments (concentration of Mancozeb 80 WP)				
	C ₀	C ₁	C ₂	C ₃	C ₄
ICM1	100.00 b	0.00 a	0.00 a	0.00 a	0.00 a
ICM2	100.00 b	0.00 a	0.00 a	0.00 a	0.00 a
ICM3	94.17 b	0.00 a	0.00 a	0.00 a	0.00 a
ICM4	94.17 b	0.00 a	0.00 a	0.00 a	0.00 a
ICM5	90.83 b	0.00 a	0.00 a	0.00 a	0.00 a
ICK1	99.17 b	0.00 a	0.00 a	0.00 a	0.00 a
ICK2	95.00 b	0.00 a	0.00 a	0.00 a	0.00 a
ICK3	94.17 b	0.00 a	0.00 a	0.00 a	0.00 a
ICK4	95.00 b	0.00 a	0.00 a	0.00 a	0.00 a
ICK5	90.83 b	0.00 a	0.00 a	0.00 a	0.00 a

Values followed by the same letters in the same column are not statistically different at the 5% threshold according to Duncan's test



Fig. 2. Impact of Mancozeb on plants inoculated with spores of *Colletotrichum gloeosporioides* after 14 days. (a) plants treated with mancozeb 5 mg/ml; (b) plants treated with mancozeb 0.005 mg/ml; (c) plants not treated C₀ = 0 mg/ml

most effective against anthracnose caused by *Euonymus fortunei*. [41,42,44]. Showed that the uses of Mancozeb 80 WP and Carbendazime were effective in reducing anthracnose due to *Colletotrichum lindemuthianum* in bean fields and mango anthracnose infections due to *C. gloeosporioides*.

Several previous studies have revealed the efficacy of Mancozeb 80 WP on other pathogenic fungi. [34,45]. had shown that Mancozeb was very effective in reducing the development of amaranth canker caused by *Phytophthora* sp, cercosporiosis caused by *Cercospora beticola* on lettuce and the incidence of late blight on potatoes due to *P. infestans*.

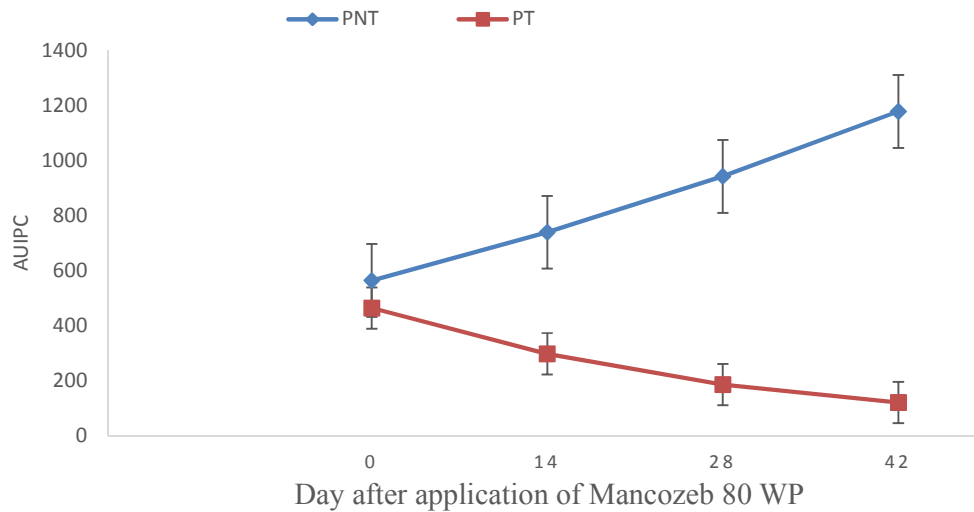


Fig. 3. Evolution of anthracnose incidence expressed as the area under the disease incidence progression curve (AUIPC) on cashew plants treated with Mancozeb 80 WP for 56 days at the Kélo site. (PT= plants treated; PNT= plants no treated)

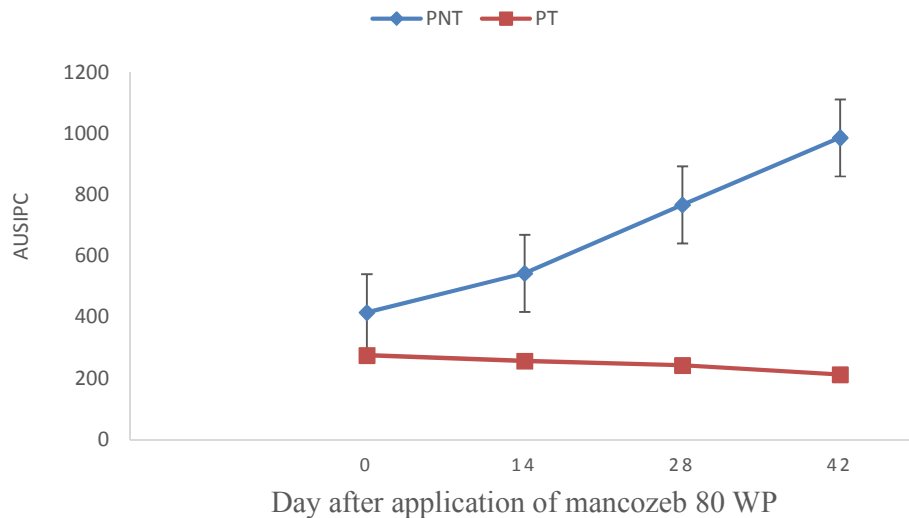


Fig. 4. Evolution of anthracnose severity expressed as the area under the disease severity index progression curve (AUSiPC) on cashew plants treated with Mancozeb 80 WP for 56 days at the Kélo site. (PT= plants treated; PNT= plants no treated)

5. CONCLUSION

Mancozeb 80 WP has been shown to be highly effective both in reducing the growth and germination of *C. gloeosporioides* spores *in vitro* and in protecting cashew nut plants *in vivo*. The application of 5 g/l of Mancozeb in the field

significantly reduced the incidence and severity of treated plants. Therefore, it would be necessary to advise nurserymen and growers to use this fungicide for the protection of young and old cashew plants to ensure yield in plantations and to increase the proportion of marketable cashew fruit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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