

Antifungal activity of some inorganic salts on the development of *Fusarium* spp., causal agents of potato tuber dry rot

Abstract

Potato dry rot caused by *Fusarium* spp. is one of the main fungal diseases hampering the production and marketing of potato tubers in Cameroon. This study was carried out in order to improve the health status of potato tubers and their preservation. To achieve this objective, *Fusarium* spp. were isolated on the Potato Dextrose Agar (PDA) culture medium and identified on the basis of morphological and microscopic characteristics using reference documents. Antifungal activity of six inorganic salts was evaluated *in vitro* on the development of *Fusarium* spp. and on potato tubers artificially infected with these fungal species. The results showed that all the inorganic salts had a depressant effect on the development of *Fusarium* spp. Copper sulfate inhibited at 100% the radial growth and sporulation of *Fusarium* spp. at all concentrations. Sodium metabisulfite and sodium carbonate inhibited at 100% the growth and sporulation of all fungal species from a concentration of 6.4 mg/ml. No lesions were observed on inoculated potato tubers when they were treated with copper sulfate. These results suggest the implementation of a strategy to control dry rot of potato tubers through the use of inorganic salts, in order to reduce the losses caused by ~~them~~ *Fusarium* spp.

Key words: Potato dry rot, *Fusarium* spp., Inorganic salts, Antifungal activities, isolates

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Introduction

Potato (*Solanum tuberosum* L.) is an annual plant of the Solanaceae family native to South America [1]. It is widely cultivated in many regions of the world for its high-energy tubers, which are rich in carbohydrates, fatty acids, proteins, vitamins and minerals [2]. Its high content of bioactive compounds such as glycoalkaloids, terpenoids and phenolic compounds makes potatoes a good antioxidant. Regular consumption of raw potato juice relieves gastric pain and helps ulcers heal by reducing stomach acidity [3]. Within the Solanaceae family, the potato is one of the world's most important food and cash crop. It is one of the main crops essential to insure food security [4].

With global production of around 368.2 million tons in 2018, potatoes remain the world's third most important food crop after rice and wheat. In Africa, Cameroon with the

production estimated at 47000 tons, was the 15th largest potato producer country and 1st in Central Africa [5]. Despite the rank he occupies, potato production in Cameroon is steadily increasing, and its cultivation occupies an important place in the national economy. The North-West and West regions are the main potato production spots, with over 85% of national production (39950 tons)[5]. With an annual consumption of about 4-10 kg/inhabitant, potato tubers cultivation is not only an important source of food for the local population but also an important source of income for producers, as extra production is sold in local markets or exported to neighboring countries [6].

Despite its socio-economic importance, this crop faces a number of abiotic and biotic constraints. Among the biotic constraints, fungal diseases are the most important. This fungal disease includes dry rot, caused by *Fusarium* spp. This disease is generally observed during storage, and manifests itself through browning of the tuber epidermis; affected tissues turn brown and become depressed, and may even show concentric striations. The tuber may progressively dry out to give a "mummified" tuber of hard consistency. If humidity is high, bacterial soft rots may add to the symptoms and cause more considerable losses for about 25% [7]. In addition to these post-harvest losses, these microorganisms also produce mycotoxins that are dangerous to human health and are responsible for many cancers [8,9].

To fight against this disease, the use of chemical fungicides is often recommended. However, these chemicals fungicides cause certain problems, such as environmental pollution, the presence of residues in the agricultural product and the development of pathogen resistance to the chemical molecules used [10]. Faced with these harmful effects of chemical fungicides, considerable effort is being devoted to exploring alternative control methods; one of which can be inorganic salts. Work by [11] showed that the inorganic salts such as potassium bicarbonate, and ammonium sodium had a strong inhibitory effect on the development of *Fusarium oxysporum*, *Alternaria alternata* and *Botrytis cinerea*, causal agents of potato dry rot, tomato leaf spot and grape gray mold, respectively. The present study was initiated with the aim to ameliorate potato tubers production through the control of ~~controlling~~ *Fusarium* spp, causal agents of dry rot by the use of inorganic salts.

Materials and methods

Description of the Study area

This study was conducted at the University of Dschang, Cameroon, in the Plant Pathology and Agricultural Zoology Research Unit. Dschang is located at an altitude of 1400m, between 5°27'0" North latitude and 10°4'0" East longitude. The prevailing climate is

equatorial with a mean annual temperature oscillating between 18.9°C and 21.1°C, a relative humidity of 90% and mean annual rainfall of 1566.8 mm [12,13].

Materials

Collection of Potato tuber

Four potato varieties (Awousang, Bango, Desire and Dosa) showing [typical](#) symptoms of dry rot and those [apparently healthy](#) were collected from potato growers in the locality of Dschang, West region of Cameroon. [These potato tubers](#) ~~These potato of each variety~~ [were](#) replaced in appropriate labeled plastic bags and transported to the Plant Pathology and Agricultural Zoology Research Unit of the University of Dschang for isolation of *Fusarium* spp.

Inorganic salts

Six inorganic salts (Calcium carbonate, calcium chloride, sodium bicarbonate, sodium carbonate, sodium metabisulfite and copper sulfate) were used for this study. All the six inorganic salts were purchased from a firm selling laboratory chemicals at Buea.

Methods

Preparation of inorganic salt solutions

The stock solutions of the inorganic salts at concentrations of 64, 128 and 256 mg/ml were prepared by soaking for each salt with volume 6.4, 12.8 and 25.6 g, respectively in 100 ml of sterile distilled water. Subsequently, 1 ml of the different stock solutions was introduced in 19 ml of medium of PDA. This made it possible to have respective concentrations of 3.2, 6.4 and 12.8 mg/ml. These ~~latter~~ concentrations were ~~those then~~ used for the test in vitro [of](#) the antifungal activity [with](#) the inorganic salts.

Isolation of *Fusarium* spp. associated with dry rot of potato tubers

Once at the research unit, tubers showing symptom of dry rot were washed separately with tap water. Fragments of necrotic tissue measuring around 2 mm² were removed from various tubers using a sterilized scalpel. The surface of the fragments was disinfected with 5% sodium hypochlorite solution for 2 minutes, followed by 3 rinses with sterilized distilled water. These fragments were aseptically seeded under a microbiological hood near the flame of a Bunsen burner in Petri dishes containing 20 ml of PDA medium (supplemented with 1 g of Chloramphenicol per liter to prevent bacterial growth) at a rate of 5 fragments per Petri dish and placed in the dark in an incubator at a temperature of 24 ± 2° C [14].

After 2 to 3 days of incubation, the fungal colonies visible around the seeded fragments were sub-cultured on fresh PDA medium until pure cultures were obtained. In this way, the cultures of different isolates were obtained and maintained in a refrigerator at 4° C. Morphological identification of *Fusarium* isolates was carried out based on the cultural characteristics and with the help of identification keys of mycology [13,15,16]. The list of different morphotypes used in this study is given in the table 1.

Table 1: Origin of *Fusarium* spp. associated with potato dry rot and used in this study

| Morphotype code | Host variety potato |
|-----------------|---------------------|
| FO1 | Awousang |
| FO2 | Banso |
| FO3 | Desire |
| FS | Dosa |

***In vitro* antifungal activity of inorganic salts on the growth of *Fusarium* spp.**

The antifungal activity of inorganic salts on the radial growth of different morphotypes of *Fusarium* spp. was assessed using the solid-state dispersion method. The different inorganic salts were tested at concentration of 3.2 mg/ml, 6.4 mg/ml and 12.8 mg/ml. The choice of the different concentrations was made on the basis of the results of the preliminary tests. These concentrations were obtained by adding 1ml of each of the previously prepared dilutions of inorganic salt to 19 ml of PDA medium. This culture medium was poured into 90 mm diameter Petri dishes. Petri dishes devoid of inorganic salts, and having received 1 ml of distilled water or thiabendazole at the manufacturer's dose (0.33 mg/ml), served respectively as negative and positive controls [14]. After solidification of the PDA medium, an explant of mycelium was removed from the growth front of the pure culture of the different 10-day-old morphotypes of *Fusarium* spp. using a 5 mm diameter punch, and then aseptically deposited in the center of each Petri dish. The Petri dishes were incubated at 24 ± 2° C for 10 days (the time required for fungal colony development in the negative control Petri containers to reach the periphery). This experiment was carried out in a completely randomized set-up with 3 repetitions. Radial growth of the different morphotypes of *Fusarium* spp. was assessed by daily measurements of the two orthogonal diameters plotted on the reverse side of the Petri containers. Radial growth data were transformed into radial growth inhibition (PI) percentages using the following formula:

$$PI (\%) = \frac{DT - D}{DT} \times 100$$

With DT and D being the radial growth diameter of the supplemented and negative control Petri dishes respectively.

These percentages of radial growth inhibition of the fungi tested were transformed into probits to determine the concentrations equivalent to 50 and 90 percent radial growth inhibition (EC₅₀ and EC₉₀).

Sporulation inhibition percentages were determined by the following method: Petri dishes in which *Fusarium* spp. growth was observed were carefully brushed with a fine brush in 20 ml sterilized distilled water. The resulting conidial suspension was stirred with a vortex to release the conidia (macroconidia and microconidia) from the conidiophores, then this suspension was filtered (to remove mycelial fragments) and a drop of Tween 20 was added to homogenize the suspension. Finally the number of conidia/ml was counted using a Thoma cell [17]. The percentage of sporulation inhibition was determined by the following formula:

$$Is (\%) = \frac{N0 - Nc}{N0} \times 100$$

With: N₀: the average number of conidia estimated in the negative control and N_c: the average number of conidia estimated in the presence of the salt.

To assess the nature of inorganic salt toxicity, we took mycelial explants that had been completely inhibited in PDA medium supplemented with various inorganic salt solutions, aseptically deposited them separately on a new PDA medium devoid of inorganic salt solution and incubated them at a temperature of 24 ± 2° C. On the fifth day after incubation, the action of the salt was considered fungicidal insofar as there was no resumption of growth of the fungal species, and fungistatic in the opposite case, i.e. resumption of growth of the fungus.

Antifungal activity of inorganic salts on the development of *Fusarium* spp. inoculated on potato tubers

Preparation of inoculum

The inoculum was prepared from a 10-day-old pure culture of *Fusarium* spp. The conidial suspension was obtained by brushing the pure culture of each fungus with 20 ml of sterilized distilled water using a fine brush. After filtration (to remove mycelial fragments), a drop of Tween 20 was added to homogenize the suspension. Inocula were quantified using a Thoma cell at 5 x 10⁶ conidia/ml [14].

Preparation of potato tubers

Apparently healthy potato tubers were washed separately with tap water, dried and then superficially disinfected with 70° alcohol.

Inoculation of potato tubers

Two methods were used.

First method

The method involved creating 5 mm-diameter openings on the potato tubers using a 5 mm-diameter punch. 25 µl of solutions of the various inorganic salts together with 25 µl of *Fusarium* spp. inoculum 5×10^6 conidia/ml were aseptically transferred simultaneously inside these openings. The edges of the openings were sealed with petroleum jelly to prevent other microorganisms from entering. Tubers receiving inoculum and thiabendazole solution at the manufacturer's dose (0.33 mg/ml) and those receiving inoculum only served as positive and negative controls respectively [14]. These tubers were placed in a sterilizer and the whole set was incubated at ambient laboratory temperature (20-22° C) for 10 days. After 10 days of incubation, the lesion area developed by each *Fusarium* spp. on the different potato tubers was measured using graph paper. The experiment followed a completely randomized design with three repetitions.

Second method

Potato tubers were sprayed simultaneously with 5 ml solution of the various inorganic salts and 5 ml inoculum of *Fusarium* spp. 5×10^6 conidia/ml [14]. These tubers were placed in a sterilizer and the whole set was incubated at ambient laboratory temperature (20-22° C) for 10 days. After 10 days of incubation, the lesion area developed by each *Fusarium* spp. on the various potato tubers was measured using graph paper. The experiment followed a completely randomized design with three repetitions.

Statistical analysis of data

Data collected on percentage radial growth inhibition, percentage sporulation inhibition, lesion area, EC_{50} and EC_{90} were subjected to analysis of variance (ANOVA) using SPSS version 22.0 software. Means were separated using Duncan's Test at the 5% probability level.

Results

Effect of inorganic salts *in vitro* on the growth of different *Fusarium* spp.

The various inorganic salts all showed a depressive effect on the radial growth of *Fusarium* spp. In general, this depressive effect varied and depended on salt type, *Fusarium* morphotype and salt concentration applied. An increase in the salt concentration of the solution led to an increase in the percentage of radial growth inhibition of the various *Fusarium* spp. (Table 2). With the exception of petri dishes treated with calcium carbonate and calcium chloride, all Petri dishes showed a higher percentage of radial growth inhibition than positive control. sodium carbonate, sodium bicarbonate and sodium metabisulfite at concentrations of 6.4 mg/ml and above completely inhibited radial growth of all *Fusarium* spp. 100% inhibition of radial growth of all *Fusarium* spp. was achieved with copper sulfate at all concentrations. In the case of calcium carbonate and calcium chloride, the radial growth inhibition percentages obtained were significantly lower than those of the positive control. Inhibition percentages ranged from 1.96 to 30.19%.

Table 2: Percentage inhibition (%) of radial growth of *Fusarium* spp. by different inorganic salts

| | Conc.(mg/ml) | FO1 | FO2 | FO3 | FS |
|-------------------|---------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Calcium carbonate | 0 (T-) | 0,00±0,00 ^d | 0,00±0,00 ^d | 0,00±0,00 ^e | 0,00±0,00 ^d |
| | 3,2 | 5,09±1,79 ^c | 4,78±0,68 ^c | 6,51±0,76 ^d | 1,96±1,77 ^d |
| | 6,4 | 8,23±3,11 ^c | 6,66±0,67 ^c | 13,72±1,35 ^c | 8,23±1,17 ^c |
| | 12,8 | 20,78±1,79 ^b | 22,35±3,52 ^b | 30,19±1,79 ^b | 24,71±4,70 ^b |
| | 0,33 (T+) | 52,54±3,59 ^a | 51,76±2,35 ^a | 59,21±1,45 ^a | 58,43±1,13 ^a |

| | | | | | |
|----------------------|-----------|--------------------------|--------------------------|--------------------------|--------------------------|
| Calcium chloride | 0 (T-) | 0,00±0,00 ^d | 0,00±0,00 ^c | 0,00±0,00 ^d | 0,00±0,00 ^d |
| | 3,2 | 10,98±1,79 ^c | 6,12±0,66 ^d | 10,58±2,96 ^c | 3,13±0,96 ^c |
| | 6,4 | 14,11±3,22 ^c | 12,54±1,68 ^c | 15,680±0,01 ^c | 7,05±1,17 ^c |
| | 12,8 | 26,66±1,79 ^b | 28,23±3,52 ^b | 30,19±1,41 ^b | 15,68±2,96 ^b |
| | 0,33 (T+) | 52,54±3,59 ^a | 51,76±2,35 ^a | 59,21±1,45 ^a | 58,43±1,13 ^a |
| Sodium bicarbonate | 0 (T-) | 0,00±0,00 ^d | 0,00±0,00 ^d | 0,00±0,00 ^d | 0,00±0,00 ^d |
| | 3,2 | 81,56±0,55 ^b | 34,11±3,52 ^c | 83,13±2,71 ^b | 76,07±1,35 ^b |
| | 6,4 | 95,68±1,47 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 95,29±0,15 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 52,54±3,59 ^c | 51,76±2,35 ^b | 59,21±1,45 ^c | 58,43±1,13 ^c |
| Sodium carbonate | 0 (T-) | 0,00±0,00 ^c | 0,00±0,00 ^d | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 3,2 | 58,82±0,33 ^b | 58,03±2,96 ^b | 54,90±3,22 ^b | 60,78±1,31 ^b |
| | 6,4 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 52,54±3,59 ^b | 51,76±2,35 ^c | 59,21±1,45 ^b | 58,43±1,13 ^b |
| Sodium metabisulfite | 0 (T-) | 0,00±0,00 ^d | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^d |
| | 3,2 | 62,54±3,59 ^b | 54,50±3,39 ^b | 60,39±1,12 ^b | 66,27±0,00 ^b |
| | 6,4 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 52,54±3,59 ^c | 51,76±2,35 ^b | 59,21±1,45 ^b | 58,43±1,13 ^c |
| Copper sulfate | 0 (T-) | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 3,2 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 6,4 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 52,54±3,59 ^b | 51,76±2,35 ^b | 59,21±1,45 ^b | 58,43±1,13 ^b |

*Values in the same column followed by different letters are significantly different ($p \leq 0.05$) according to Duncan Multiple Range Test. T- = negative control (Distilled water); T+ = positive control (Thiabendazole)

The values for concentrations equivalent to 50 and 90% inhibition by inorganic salts 10 days after transplanting the various *Fusarium* spp. were quite varied (Table 3). With morphotypes FO1 and FO2, calcium carbonate showed the highest EC₅₀ and CE₉₀. These EC₅₀ were 25.37 and 22.93 mg/ml for F1DS and FO2 morphotypes respectively. CE₉₀ values were 56.12 mg/ml for FO1 morphotypes and 52.44 mg/ml for F2DS morphotypes. The lowest EC₅₀ and CE₉₀ values were observed with sodium carbonate and sodium metabisulfite. These values ranged from 2.88 to 3.18 mg/ml for EC₅₀ and from 5.37 to 6.01 mg/ml for EC₉₀. For the FO3 morphotype, the EC₉₀ values for calcium carbonate and calcium chloride were significantly identical to and higher than those for the other inorganic salts, according to

Duncan's 5% test. These EC₉₀ values were 46.55 and 45.90 mg/ml respectively. sodium carbonate was the salt with the lowest EC₉₀ value (3.79 mg/ml). Copper sulfate EC₅₀ and EC₉₀ values were not determined, as it completely inhibited radial growth of all *Fusarium* spp. at all concentrations.

Table 3: EC₅₀ and EC₉₀ values (mg/ml)

| | FO1 | FO2 | FO3 | FS |
|----------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| CE ₅₀ | | | | |
| Calcium carbonate | 25,37±0,33 ^a | 22,93±0,55 ^a | 19,54±0,40 ^b | 20,54±0,40 ^b |
| Calcium chloride | 23,31±0,31 ^b | 21,87±0,45 ^b | 20,75±0,56 ^a | 29,45±1,95 ^a |
| Sodium bicarbonate | 1,11±0,08 ^d | 1,02±0,05 ^c | 1,05±0,04 ^c | 1,46±0,15 ^c |
| Sodium carbonate | 3,06±0,06 ^c | 3,14±0,03 ^c | 3,58±0,08 ^c | 2,92±0,07 ^c |
| Sodium metabisulfite | 2,88±0,09 ^c | 3,18±0,16 ^c | 2,93±0,11 ^d | 2,60±0,15 ^c |
| Copper sulfate | / | / | / | / |
| CE ₉₀ | | | | |
| Calcium carbonate | 56,12±0,52 ^a | 52,44±1,38 ^a | 46,55±0,50 ^a | 51,39±2,55 ^b |
| Calcium chloride | 53,80±0,41 ^b | 51,04±0,94 ^b | 45,90±0,81 ^a | 59,74±1,40 ^a |
| Sodium bicarbonate | 4,51±0,22 ^d | 3,51±0,17 ^d | 3,79±0,23 ^c | 4,58±0,13 ^c |
| Sodium carbonate | 5,37±0,13 ^c | 5,73±0,05 ^c | 6,13±0,06 ^b | 5,20±0,07 ^c |
| Sodium metabisulfite | 5,57±0,09 ^c | 6,01±0,02 ^c | 5,49±0,03 ^b | 5,22±0,05 ^c |
| Sulfate decuivre | / | / | / | / |

*Values in the same column followed by different letters are significantly different ($p \leq 0.05$) according to Duncan Multiple Range Test. / means that the EC₅₀ or EC₉₀ value has not been determined.

Percentage inhibition of *Fusarium* spp. sporulation varied according to inorganic salt type, salt concentration applied and *Fusarium* spp. morphotype (table 4). Petri dishes treated with sodium bicarbonate at concentrations of 3.2 and 6.4 mg/ml on FO1 morphotypes showed sporulation inhibition percentages of 66.93 and 96.05% respectively. With FS morphotypes, at the same concentrations, these inhibition percentages were 71.66 and 94.96% respectively. Copper sulfate, at all concentrations, inhibited sporulation of the various *Fusarium* spp. to 100%. The same finding was made with sodium carbonate and sodium metabisulfite at concentrations of 6.4 and 12.8 mg/ml.

Table 4: Percentage inhibition (%) of *Fusarium* spp. sporulation by different inorganic salts

| | Conc.(mg/ml) | FO1 | FO2 | FO3 | FS |
|-----------|--------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Calcium | 0 (T-) | 0,00±0,00 ^e | 0,00±0,00 ^d | 0,00±0,00 ^d | 0,00±0,00 ^e |
| carbonate | 3,2 | 13,33±1,56 ^d | 4,29±2,46 ^d | 17,98±1,28 ^c | 6,63±2,77 ^d |
| | 6,4 | 22,82±2,16 ^c | 15,64±1,43 ^c | 20,34±4,84 ^c | 12,50±1,07 ^e |
| | 12,8 | 63,13±1,39 ^b | 30,19±1,39 ^b | 34,61±1,27 ^b | 25,29±3,21 ^b |
| | 0,33 (T+) | 78,23±3,03 | 71,29±2,05 ^a | 82,29±0,51 ^a | 48,60±2,65 ^a |
| Calcium | 0 (T-) | 0,00±0,00 ^e | 0,00±0,00 ^d | 0,00±0,00 ^e | 0,00±0,00 ^e |

| | | | | | |
|---------------|-----------|--------------------------|--------------------------|--------------------------|--------------------------|
| chloride | 3,2 | 4,65±1,90 ^d | 9,48±3,93 ^d | 33,57±1,25 ^d | 6,99±2,01 ^d |
| | 6,4 | 14,98±3,08 ^c | 34,21±0,85 ^c | 48,00±1,51 ^c | 17,08±1,00 ^c |
| | 12,8 | 31,03±1,52 ^b | 50,05±3,38 ^b | 61,57±1,60 ^b | 26,94±1,51 ^b |
| | 0,33 (T+) | 78,23±3,03 ^a | 71,29±2,05 ^a | 82,29±0,51 ^a | 48,60±2,65 ^a |
| | Sodium | 0 (T-) | 0,00±0,00 ^e | 0,00±0,00 ^d | 0,00±0,00 ^d |
| bicarbonate | 3,2 | 66,93±2,44 ^d | 48,35±2,83 ^c | 77,81±1,73 ^c | 71,65±1,54 ^b |
| | 6,4 | 96,05±0,43 ^b | 100,00±0,00 ^a | 100,00±0,00 ^a | 94,96±1,09 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 78,23±3,03 ^c | 71,29±2,05 ^b | 82,29±0,51 ^b | 48,60±2,65 ^c |
| | Sodium | 0 (T-) | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| carbonate | 3,2 | 75,44±2,44 ^b | 68,96±0,89 ^b | 83,17±3,85 ^b | 70,91±3,56 ^b |
| | 6,4 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 78,23±3,03 ^b | 71,29±2,05 ^b | 82,29±0,51 ^b | 48,60±2,65 ^c |
| | Sodium | 0 (T-) | 0,00±0,00 ^d | 0,00±0,00 ^d | 0,00±0,00 ^c |
| metabisulfite | 3,2 | 74,02±0,70 ^c | 64,53±1,57 ^c | 80,99±1,53 ^b | 53,17±3,36 ^b |
| | 6,4 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 78,23±3,03 ^b | 71,29±2,05 ^b | 82,29±0,51 ^b | 48,60±2,65 ^c |
| | Sodium | 0 (T-) | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| sulfate | 3,2 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 6,4 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 12,8 | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a | 100,00±0,00 ^a |
| | 0,33 (T+) | 78,23±3,03 ^b | 71,29±2,05 ^b | 82,29±0,51 ^b | 48,60±2,65 ^b |

Values in the same column followed by different letters are significantly different ($p \leq 0.05$) according to Duncan Multiple Range Test. T- = negative control (Distilled water); T+ = positive control (Thiabendazole)

Nature of fungitoxicity of inorganic salts on *Fusarium* spp.

All the inorganic salts at the concentrations which were presented 100% of inhibition of radial growth of different morphotypes of *Fusarium* spp., showed a fungicide activity.

Effect of inorganic salts on the development of *Fusarium* spp. inoculated on potato tubers

In order to assess the effect of inorganic salts on the development of *Fusarium* spp. inoculated on potato tubers, salts that had shown significant *in vitro* efficacy on *Fusarium* spp. were used. These included sodium bicarbonate and sodium carbonate at concentrations of

6.4mg/ml and 12.8mg/ml, and sodium metabisulfite and copper sulfate at all concentrations (3.2mg/ml, 6.4mg/ml and 12.8mg/ml). Lesion areas induced by *Fusarium* spp. morphotypes varied according to the inorganic salt applied, the concentration used, the morphotype to which the salt was applied and the inoculation method. The higher the concentration of salt solution applied, the smaller the lesion area induced by the different *Fusarium* spp. Nevertheless, tubers treated with inorganic salts showed smaller lesion areas than the negative control

Table 5 shows the lesion areas induced by *Fusarium* spp. 10 days after simultaneous deposition of their inoculum and inorganic salt solution at different concentrations on opened potato tubers. The negative control potato tubers showed larger lesion areas than the other potato tubers. Lesion areas induced by F4DS morphotypes were smaller than those induced by other *Fusarium* spp. regardless of inorganic salt or concentration applied. Potato tubers treated with calcium carbonate and calcium bicarbonate at concentrations of 6.4 mg/ml and 12.8 mg/ml showed the largest lesion areas compared with the other salts. With sodium metabisulfite, the smallest lesion areas were observed at a concentration of 12.8 mg/ml. These induced lesion areas were 11.33, 13.87, 11.33 and 11.00 mm² for FO1, FO2, FO3 and FS morphotypes respectively. Regarding copper sulfate, no lesion area was induced by *Fusarium* spp. regardless of concentration and morphotype of *Fusarium* spp.

Table 5: Lesion area (mm²) induced by *Fusarium* spp. on injured potato tubers

| | Conc.(mg/ml) | FO1 | FO2 | FO3 | FS |
|-------------|--------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Sodium | 0 (T-) | 113,33±0,57 ^a | 110,33±1,52 ^a | 104,12±1,50 ^a | 96,00±1,00 ^a |
| bicarbonate | 6,4 | 101,33±2,30 ^b | 102,66±3,78 ^a | 96,18±1,52 ^b | 85,23±0,78 ^a |
| | 12,8 | 91,61±1,52 ^c | 96,21±1,05 ^a | 82,30±2,51 ^c | 63,31±1,38 ^b |
| | 0,33 (T+) | 47,66±0,64 ^d | 58,31±0,48 ^b | 31,00±1,00 ^d | 28,16±1,52 ^c |
| Sodium | 0 (T-) | 113,33±0,57 ^a | 110,33±1,52 ^a | 104,12±1,50 ^a | 96,00±1,00 ^a |

| | | | | | |
|----------------|-----------|--------------------------|--------------------------|--------------------------|-------------------------|
| carbonate | 6,4 | 95,16±1,93 ^a | 101,66±1,52 ^a | 98,00±1,00 ^b | 83,17±1,05 ^b |
| | 12,8 | 94,18±0,37 ^a | 96,00±0,35 ^a | 74,12±0,21 ^c | 65,09±2,35 ^c |
| | 0,33 (T+) | 47,66±0,64 ^b | 58,31±0,48 ^b | 31,00±1,00 ^d | 28,16±1,52 ^d |
| Sodium | 0 (T-) | 113,33±0,57 ^a | 110,33±1,52 ^a | 104,12±1,50 ^a | 96,00±1,00 ^a |
| metabisulfite | 3,2 | 72,67±0,02 ^b | 76,67±1,66 ^b | 67,67±0,57 ^b | 65,07±1,58 ^b |
| | 6,4 | 31,29±3,05 ^d | 41,21±1,52 ^c | 33,67±1,65 ^c | 11,29±0,63 ^d |
| | 12,8 | 11,33±1,52 ^e | 13,87±1,51 ^d | 11,33±0,29 ^d | 11,00±1,00 ^d |
| | 0,33 (T+) | 47,66±0,64 ^c | 58,31±0,48 ^c | 31,00±1,00 ^c | 28,16±1,52 ^c |
| Copper sulfate | 0 (T-) | 113,33±0,57 ^a | 110,33±1,52 ^a | 104,12±1,50 ^a | 96,00±1,00 ^a |
| | 3,2 | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 6,4 | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 12,8 | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 0,33 (T+) | 47,66±0,64 ^b | 58,31±0,48 ^b | 31,00±1,00 ^b | 28,16±1,52 ^b |

Values in the same column followed by different letters are significantly different ($p \leq 0.05$) according to Duncan Multiple Range Test. T- = negative control (Distilled water); T+ = positive control (Thiabendazole)

The spray-inoculation method induced fairly low lesion areas (Table 6). Potato tubers inoculated with the FS morphotype and treated with sodium carbonate and sodium bicarbonate at a concentration of 12.8 mg/ml showed significantly lower lesion areas than those inoculated with the other morphotypes of *Fusarium* spp. These lesion areas were 11.00 mm². Potato tubers treated with sodium metabisulfite at concentrations of 6.4 and 12.8 mg/ml showed smaller lesion areas than the positive and negative controls. These lesion areas ranged from 6.54 to 14.09 mm². No lesion surfaces were observed on potato tubers treated with copper sulfate, whatever the inoculum. The inoculum prepared from the FS morphotype produced significantly lower lesion areas than those prepared from the other morphotypes.

Table 6: Lesion area (mm²) induced by *Fusarium* spp. on potato tubers following spray inoculation

| | Conc. (mg/ml) | FO1 | FO2 | FO3 | FS |
|-------------|---------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Sodium | 0 (T-) | 57,80±1,98 ^a | 61,39±1,44 ^a | 43,81±1,52 ^a | 38,65±0,40 ^a |
| bicarbonate | 6,4 | 44,33±1,05 ^b | 41,91±1,50 ^b | 36,29±2,08 ^b | 24,68±0,21 ^b |
| | 12,8 | 32,21±0,52 ^c | 32,00±3,46 ^c | 21,00±1,00 ^c | 15,32±0,82 ^c |
| | 0,33 (T+) | 22,00±1,73 ^d | 26,66±1,52 ^d | 17,33±0,57 ^d | 11,00±1,00 ^d |

| | | | | | |
|----------------------|-----------|-------------------------|-------------------------|-------------------------|-------------------------|
| Sodium carbonate | 0 (T-) | 57,80±1,98 ^a | 61,39±1,44 ^a | 43,81±1,52 ^a | 38,65±0,40 ^a |
| | 6,4 | 44,66±2,51 ^b | 42,13±1,15 ^b | 36,00±1,00 ^b | 33,12±2,00 ^b |
| | 12,8 | 35,32±1,52 ^c | 36,33±2,08 ^c | 24,71±3,51 ^c | 18,24±0,57 ^c |
| | 0,33 (T+) | 22,00±1,73 ^d | 26,66±1,52 ^d | 17,33±0,57 ^d | 11,00±1,00 ^d |
| | 0 (T-) | 57,80±1,98 ^a | 61,39±1,44 ^a | 43,81±1,52 ^a | 38,65±0,40 ^a |
| Sodium metabisulfite | 3,2 | 24,91±2,51 ^b | 24,45±2,88 ^b | 23,16±1,73 ^b | 19,27±0,21 ^b |
| | 6,4 | 12,00±1,73 ^c | 14,09±3,00 ^c | 10,80±0,59 ^d | 9,00±0,01 ^d |
| | 12,8 | 9,12±0,82 ^d | 11,62±1,44 ^c | 7,13±0,23 ^e | 6,54±0,64 ^e |
| | 0,33 (T+) | 22,00±1,73 ^b | 26,66±1,52 ^b | 17,33±0,57 ^c | 11,00±1,00 ^c |
| | 0 (T-) | 57,80±1,98 ^a | 61,39±1,44 ^a | 43,81±1,52 ^a | 38,65±0,40 ^a |
| Copper sulfate | 3,2 | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 6,4 | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 12,8 | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c | 0,00±0,00 ^c |
| | 0,33 (T+) | 22,00±1,73 ^b | 26,66±1,52 ^b | 17,33±0,57 ^b | 11,00±1,00 ^b |
| | 0 (T-) | 57,80±1,98 ^a | 61,39±1,44 ^a | 43,81±1,52 ^a | 38,65±0,40 ^a |

Values in the same column followed by different letters are significantly different ($p \leq 0.05$) according to Duncan Multiple Range Test. T- = negative control (Distilled water); T+ = positive control (Thiabendazole)

Discussion

Efficacy of inorganic salts *in vitro* on the growth of different morphotypes of *Fusarium* spp. associated with potato tubers

The various inorganic salts have shown an overall depressive effect on the radial growth of *Fusarium* spp. compared with the negative control. This depressive effect may be due to the fact that the inorganic salts used contain molecules with antifungal properties capable of inhibiting the growth of the fungal species tested. Indeed, numerous studies have reported that inorganic salts used in the food industry as preservatives possess bactericidal or fungicidal activity that can be exploited for phytosanitary purposes [18,19,20].

The percentages of inhibition of radial growth of *Fusarium* spp. varied according to the inorganic salt used, the concentration applied and the morphotype of *Fusarium* spp. The percentage of inhibition of the different *Fusarium* spp. increased with rising concentration, which could evoke the idea that higher concentrations of inorganic salt would be more fungicidal compared to lower concentrations. Similar results were reported by [21], who showed that radial growth of *Botrytis cinerea*, the causal agent of tomato fruit rot, was more inhibited by sodium metabisulfite, sodium bicarbonate and calcium chloride when concentrations applied were high. Sodium carbonate and sodium metabisulfite at concentrations of 6.4 mg/ml and above completely inhibited radial growth of all

Fusarium spp. These results corroborate those of [22], who showed that Ammonium bicarbonate, Ammonium carbonate, potassium benzoate, sorbate potassium, sodium carbonate and sodium metabisulfite inhibited the development of *Fusarium oxysporum*: causal agent of *Fusarium* wilt of eggplant. Similarly, those of [23,24], showed that salts such as ammonium phosphate dibasic, sodium benzoate, potassium sorbate, sodium bicarbonate, sodium carbonate and sodium metabisulfite inhibited 100% of the radial growth of various phytopathogenic agents (*Sclerotium rolfsii*, *Aspergillus niger*, *Fusarium* spp, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Rhizoctonia solani*).

Calcium carbonate and calcium chloride were unable to inhibit 100% of the radial growth of *Fusarium* spp. These results are similar to those of [21], who reported a weak inhibitory action of these two inorganic salts on the development of *Botrytis cinerea*, responsible for rotting tomato fruit. A difference in the inhibitory action of these salts was observed with other authors. This difference in results may be due to the fact that *Fusarium* spp. are less sensitive to calcium carbonate and calcium chloride than to other pathogens.

EC₅₀ and EC₉₀ values were fairly heterogeneous. Sodium bicarbonate, sodium carbonate and sodium metabisulfite were significantly more effective than calcium carbonate and calcium chloride. This difference in efficiency between the inorganic salts tested could be due to the fact that some salts possess compounds with more effective antifungal properties than others.

Sporulation inhibition percentages varied according to the concentration applied, the salt and the morphotype tested. The higher the concentration applied, the greater the inhibition of *Fusarium* spp. sporulation. This may be due to the fact that, by inhibiting radial growth, the salts also simultaneously inhibit sporulation of the various fungi. Similar results were reported by [21], who showed that the greater the percentage inhibition of *Botrytis cinerea* sporulation, the lower the mycelial growth of the pathogen was.

Nature of toxicity of different salts against morphotypes of *Fusarium* spp.

All salts effective *in vitro* were fungicidal against *Fusarium* morphotypes. This could be explained by the fact that the modification of the PH of the medium by the salts would lead to the destruction of certain parts of the fungi cells or disrupt their metabolism, causing the death of the pathogen. Work carried out by [25] shows that certain ions released by salts in solution reduce the activity of the polygalacturonase enzyme frequently produced by bacteria and fungi in diseased host tissues.

Efficacy of inorganic salts on the development of different morphotypes of *Fusarium* spp. inoculated on potato tubers

The various inorganic salts slowed down the development of *Fusarium* spp. on potato tubers, whatever the inoculation method. No lesions were observed on potato tubers treated with copper sulfate. These results are similar to those of [26] and [27] who showed that certain salts completely inhibit the development of several phytopathogenic fungal species. The FS morphotype induced smaller lesion areas than those developed by the FO1, FO2 and FO3 morphotypes. This is thought to be due to the fact that FS is less virulent than other *Fusarium* spp. These results corroborate those of [14], who reported that some isolates of *Colletotrichum* spp. induced larger lesions than others when inoculated onto papaya, mango, avocado and banana fruits.

Lesions developed on potato tubers inoculated through the openings created were greater than those developed on tubers inoculated by spraying. This difference was due to the fact that the wounds created would have caused stress in the potato tubers. This would have caused a progressive reduction in the elements involved in the tuber's defense mechanism [14].

Conclusion

This study revealed that inorganic salts inhibited the radial growth of *Fusarium* spp. in vitro and significantly prevented their development when inoculated onto potato tubers. The antifungal activity of the salts was similar to that of the chemical fungicide Thiabendazole, and in some cases superior. This suggests the need for in-depth studies on the toxicity of these salts to humans and the environment, with a view to vulgarizing their practical use.

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