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 inoculated with these fungal species. The results showedthat 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. Sodium metabisulfite and sodium carbonate inhibited at100%, the growth and sporulation of all fungal species from a concentration of 6.4 mg ml^{-1}. 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 *Fusarium*spp.

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

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, terponoids 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 fungicidescause 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 *Fusariumoxysporum, Alternariaalternata*and*Botrytiscinerea*, 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 and 21.1°C, a relative humidity of 90% and mean annual rainfall of 1566.8 mm [12,13].

Materials and Materials

Collection of Potato tuber

Four potato varieties (Awousang, Banso, Desire and Dosa)showing symptoms of dry rotand those Apparently healthy were collected from potato growers in the locality of Dschang, West region of Cameroon. Potatotubers were placed 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.

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^{-1}. These latter concentrations were those used for the test in vitro the antifungal activity 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 mm^2 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 sterilizeddistilled 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 differentmorphotypes used inthis study is given in 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^{-1}. 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^{-1}), 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:

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

Where; 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 (EC_{50} and EC_{90}).

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:

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

With: No: the average number of conidia estimated in the negative control and Nc: 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 10^6 conidia ml^{-1}[14].

Preparation of potato tubers

Apparently healthy potato tubers were washed separately with tap water, dried and then superficially disinfected with 70º 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 x 10^6 conidia ml^{-1} 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 ina cristilizersand 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 x 10^6 conidia ml^{-1}[14]. These tubers were placed in crystallizersand 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 threerepetitions.

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^{-1} and above completely inhibited radial growth of all *Fusarium* spp. Complete inhibition of the 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^{-1})	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^{e}	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 morphotypesFO1 and FO2, calcium carbonate showed the highest $EC_{50}$and CE_{90}. These EC_{50} were 25.37 and 22.93 mg ml^{-1} for F1DS and FO2 morphotypesrespectively. CE_{90}values were 56.12 mg/ml for FO1morphotypesand 52.44 mg ml^{-1} for F2DSmorphotypes. The lowest EC_{50} and CE_{90} values were observed with sodium carbonate and sodium metabisulfite. These values ranged from 2.88 to 3.18 mg ml^{-1} for EC_{50} and from 5.37 to 6.01 mg ml^{-1} for EC_{90}. For the FO3 morphotype, the EC_{90} values for calcium carbonate and calcium chloride were significantly identical to and higher than those for the other inorganic salts, according to Duncan's 0.05test. These EC_{90} values were 46.55 and 45.90 mg ml^{-1},respectively. Sodium carbonate was the lowest EC_{90} value (3.79 mg ml^{-1}). Copper sulfate EC_{50} and EC_{90} values were not determined, as it completely inhibited radial growth of all *Fusarium* spp. at all concentrations.

Table 3: EC_{50} and EC_{90} 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[e]	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^{-1} on FO1morphotypesshowed sporulation inhibition percentages of 66.93 and 96.05%, respectively. With FSmorphotypes, 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^{-1}.

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

	Conc.(mg ml^{-1})	FO1	FO2	FO3	FS
Calcium carbonate	0 (T-)	0,00±0,00^{e}	0,00±0,00^{d}	0,00±0,00^{d}	0,00±0,00^{e}
	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^{c}
	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 chloride	0 (T-)	0,00±0,00^{e}	0,00±0,00^{d}	0,00±0,00^{e}	0,00±0,00^{e}
	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}	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 carbonate	0 (T-)	0,00±0,00^{c}	0,00±0,00^{c}	0,00±0,00^{c}	0,00±0,00^{d}
	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 metabisulfite	0 (T-)	0,00±0,00^{d}	0,00±0,00^{d}	0,00±0,00^{c}	0,00±0,00^{d}
	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}
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+)	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 6.4 and 12.8mg ml^{-1}, and sodium metabisulfite and copper sulfate at all concentrations (3.2, 6.4 and 12.8mg ml^{-1}). 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,

thesmaller 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 morphotypewere 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 and 12.8 mg ml^{-1} 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^{-1}. These induced lesion areas were 11.33, 13.87, 11.33 and 11.00 mm^2 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 (mm^2) induced by *Fusarium* spp. on injured potato tubers

	Conc.(mg/ml)	FO1	FO2	FO3	FS
Sodium bicarbonate	0 (T-)	113,33±0,57^a	110,33±1,52^a	104,12±1,50^a	96,00±1,00^a
	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 carbonate	0 (T-)	113,33±0,57^a	110,33±1,52^a	104,12±1,50^a	96,00±1,00^a
	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 metabisulfite	0 (T-)	113,33±0,57^a	110,33±1,52^a	104,12±1,50^a	96,00±1,00^a
	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	0 (T-)	113,33±0,57^{a}	110,33±1,52^{a}	104,12±1,50^{a}	96,00±1,00^{a}
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+)	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^{-1} showed significantly lower lesion areas than those inoculated with the other morphotypes of *Fusarium* spp. These lesion areas were 11.00 mm^2. Potato tubers treated with sodium metabisulfite at concentrations of 6.4 and 12.8 mg ml^{-1} showed smaller lesion areas than the positive and negative controls. These lesion areas ranged from 6.54 to 14.09 mm^2. 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 (mm^2) 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	0 (T-)	57,80±1,98^{a}	61,39±1,44^{a}	43,81±1,52^{a}	38,65±0,40^{a}
carbonate	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}
Sodium	0 (T-)	57,80±1,98^{a}	61,39±1,44^{a}	43,81±1,52^{a}	38,65±0,40^{a}

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}
Copper sulfate	0 (T-)	57,80±1,98^{a}	61,39±1,44^{a}	43,81±1,52^{a}	38,65±0,40^{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+)	22,00±1,73^{b}	26,66±1,52^{b}	17,33±0,57^{b}	11,00±1,00^{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)

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^{-1} and above completely inhibited radial growth of all *Fusarium* spp. These results corroborate those of [22], who showed thatAmmonium bicarbonate Ammonium carbonate, potassium benzoate, sorbate potassium, sodium carbonate and sodium metabisulfiteinhibited the development of *Fusariumoxysporum*: 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 (*Sclerotiumrolfsii*, *Aspergillusniger*, *Fusarium*spp, *Macrophominaphaseolina*, *Sclerotiumrolfsii* and *Rhizoctoniasolani*).

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.

EC_{50} and EC_{90} 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.

References

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1. Delaplace, P., Brostaux, Y., Fauconnier, M. L., andDu Jardin, P. (2008). Potato (*Solanum tuberosum* L.) tuber physiological age index is a valid reference frame in postharvest ageing studies. *Postharvest Biology and Technology*, **50,**103-106.
2. Ashenafi M. Y., Thangavel S., Alemu L. andBekele K., (2017). Evaluation of potato cultivars and fungicides for the management of Late blight (*Phytophthorainfestans*(Mont) de Bary) in Holleta, West Showa, Ethiopia. *International Journal of Life Sciences*, **5,** 161-179.
3. Umadevi, R., Kumar, S. P. K., Bowmik, D. andDuraivel, S. (2013). Health Benefits and Cons of *Solanum tuberosum*. *Journal of Medicinal Plants Studies*,**1,** 16-25.

4. Loucif, M. and Namoussa, M. (2019). Importance and distribution of cyst nematodes (Globodera) on potato cultivation (Solanum tuberosum L.) in the Wilaya of El-Oued.

5. Food and Agricultural Organization, (2019). Statistics. Food and Agriculture Organization, Rome, 33p.

6. National Roots and Tubers Development Program, (2006). Study on the roots and tubers observatory. Activity report 2005/2006. 54P.

7. Lakhel, Z., Zeghloul, Z., and Bouziane, Z.E., (2013). Contribution to the study of the aggressiveness of Fusariumoxysporum on the tubers of three potato varieties. Doctoral thesis. Jijel University.

8. Attrassi, K., Benkirane, R., Attrassi, B., and Douira, A., (2007). Effect of the association of certain fungicides with calcium chloride on the development of a fungal complex responsible for the rot of apples in storage. Phytoprotection, 88, 17-26.

9. Van Le Thanh, B., Lessard, M., Chorfi, Y., and Guay, F., (2015). The efficacy of anti-mycotoxin feed additives in preventing the adverse effects of wheat naturally contaminated with Fusarium mycotoxins on performance, intestinal barrier function and nutrient digestibility and retention in weanling pigs. Canadian Journal of Animal Science, 95, 197-209.

10. El Mehdi, A. A., El Khetabi, A., Belmalha, S., and Lahlali, R., (2021). Use of plant extracts against post-harvest fruit diseases. Moroccan Review of Agronomic and Veterinary Sciences, 9, 12-21.

11. Zaker, M., (2014). Antifungal evaluation of some inorganic salts against three phytopathogenic fungi. International Journal of Agriculture and crop sciences, 7, 1352-1357.

12. Ananfack, G., Atchombou, B., Zanguim, H.T., Awazi, N.P., Tassiamba, S.N., Tientcheu, L.T., and Tchamba, M.N., (2021). Assessing Growth Parameters from Culm Cuttings of Bambusa vulgaris at the University of Dschang Botanic Garden, West Region of Cameroon: Implications for the Bamboo Resource Base and Rural Livelihoods. Open Journal of Forestry, 12, 88-106.

13. Njimah, M.D., Tsopmbeng, N.G., Tamekou, L.S., Keuete, K.E. and Kuiate, J.R., (2021). Antifungal Activity of Some Plant Extracts Against Morphotypes of Fusarium spp. Associated with Tomato (SolanumLycopersicum L.) Fruit Rots in The West-Region of Cameroon. Journal of Food Nutrition, 7, 1-10.

14. Keuete, K.E., Tsopmbeng, N.G.R. and Kuiate, J.R., (2018). Cross-infectivity of Colletotrichumspp isolates, causal agent of anthracnose on fruits in the three regions of Cameroon. International Journal of Pure and Applied Biosciences, 6, 1-10.

15. Barnett, H.L. and Hunter, B.B., (1972). Illustrated Genera of Imperfect Fungi. Minneapolis: Burgress, Minneapolis MN, 241p.

16. Srinivas, C., Nirmala, D.D., Narasimha, K.M., Chakrabhavi, D.M., Lakshmeesha, T.R., Bhimpratap, S., Naveen K.K., Niranjana, S.R., Abeer, H., Abdulaziz, A.A., Baby, T., Elsayed , F.A.A., Chandra, S.N. and Rakesh, K.S., (2019). Fusariumoxysporum causal agent of

vascular wilt disease of tomato: Biology to diversity. Saudi journal of biological sciences, 26, 15-1324.

17. Keuete, K.E., Tsopmbeng, N.G.R. and Kuiate, J.R. (2016). Cultural and morphological variation of Colletotrichumspp associated with anthracnose of various fruits in Cameroon. International Journal of Environment, Agriculture and Biotechnology, 4, 968-974.

18. Yaganza, E.S., (2014). Post-harvest use of organic and inorganic salts to control potato soft rot: physicochemical basis. Doctoral thesis. University of Laval. 65p.

19. Türkkan, M., (2019). Effect of various salts on the growth and development of Geotrichumcandidum, the causal agent of carrot sour rot. Journal of Phytopathology, 167, 230-239.

20. Barro, A., (2022). Evaluation of different salts and extracts of forest species to combat leaf and fruit diseases in strawberries. Doctoral thesis, University of Laval.

21. Alaoui, F.T., Askarne, L., Boubaker, H., Boudyach, E. and Aoumar, A.A.B., (2017). Control of gray mold disease of tomato by postharvest application of organic acids and salts. Plant Pathology Journal, 16, 62-72.

22. Yildirim, E., Alici, E., Erper, I., Turkkan, M. and Ozer, G., (2022). Sensitivity of Fusariumoxysporum f. sp. melongenae, the causal agent of Fusarium wilt of eggplant to some ammonium, potassium and sodium compounds in vitro and in vivo bioassays. Archives of Phytopathology and Plant Protection, 55, 937-950.

23. Mansour, M., Aly, A.A., Habeb, M.M. and Mohamed, H.I., (2020). Control of Cotton Seedling Damping-off by Treating Seed with Inorganic Salts. GesundePflanzen, 72, 23-31.

24. Türkkan, M., Özcan, M. and Erper, I., (2017). Antifungal effect of carbonate and bicarbonate salts against Botrytis cinerea, causative agent of Kiwi gray mold. ZiraatDergisi Academy, 6, 107-114.

25. Delisle-Houde, M., Toussaint V. and Tweddell R.J., (2019). Evaluation of antibacterial activitybe Xanthomonascampestrispv. vitians and Pseudomonas cichorii from different plant extracts based on horticultural species and forest species. Phytoprotection, 99, 21-26.

26. Jabnoun-Khiareddine, H., Abdallah, R., El-Mohamedy, R., Abdel-Kareem, F., Gueddes-Chahed, M., Hajlaoui, A. and Daami-Remadi, M., (2016) . Comparative efficacy of potassium salts against soil-borne and air-borne fungi and their ability to suppress tomato wilt and fruit rots. Journal of Microbial and Biochemical Technology, 8, 45-55.

27. Tremblay, V., (2019). Evaluation of different plant extracts and organic and inorganic salts to combat lettuce bacterial spot. Doctoral thesis. University of Laval.