

## Original Research Article

# Antifungal activity of copper, zinc and potassium compounds on mycelial growth and conidial germination of *Fusarium solani* f. sp. *piperis*

### ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Fusariosis is a disease that causes economic damage to black pepper (*Piper nigrum* L.) producers. Despite being a major disease, there is no record of efficient chemical control. Thus, the objective was to evaluate the antifungal activity of copper, zinc and potassium compounds in mycelial growth and conidial germination of *Fusarium solani* f. sp. *piperis* *in vitro*. For inoculation in PDA (Potato Dextrose Agar) medium, 7 mm discs from the pure culture were transferred to Petri dishes. The plates were incubated at 25°C in a biochemical oxygen demand (BOD) chamber, with photoperiod of 12 h, for 15 days. Micronutrients were supplied as sulfates, CuSO<sub>4</sub> (copper sulfate) and ZnSO<sub>4</sub> (zinc sulfate), at concentrations of 1, 5, 10, 15 and 20 mmol/L. Potassium macronutrient (K) was supplied as KCl (potassium chloride) at concentrations of 30, 60, 90, 120 and 150 mmol/L. The experiment was performed using a completely randomized design with 6 treatments and ten replications. CuSO<sub>4</sub> showed fungicidal effect at concentrations of 10, 15 and 20 mmol/L. For ZnSO<sub>4</sub> mycelial growth was completely inhibited at concentrations of 15 and 20 mmol/L. There was no inhibition or reduction of fungal growth in the presence of K. Copper and zinc at minimal concentrations were efficient in controlling mycelial growth and inhibition of spore germination of *F. solani* f. sp. *piperis*. In contrast, potassium did not exert fungicidal or fungistatic effect on the fungus.

*Keywords: Fusarium solani, antifungal activity, fungal growth inhibition, nutrients*

### 1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Pathogenic fungi include a large and heterogeneous group of organisms that occupy an important position in both agriculture and natural populations [1]. The genus *Fusarium* spp. is classified as **Ascomycete**, characterized by a hyaline mycelium, branched and septate, with phyllide-shaped sporophores and conidia of varying size and shape. It has spindle-shaped macroconids with several septa [2]. *Fusarium* species are known as phytopathogens, saprophytes with worldwide distribution [3].

*Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. f. sp. *piperis*, Albuquerque (Teleomorph *Nectria haematococca* Berk. & Br. f. sp. *piperis* Albuq.) is the causal agent of fusariosis and has brought great economic damage to black pepper (*Piper nigrum* L.)

30 producers, decimating whole crops [4, 5]. The disease may start in the root system or in the  
31 shoot [6, 8]. When initiated by the roots, the root system is reduced and becomes necrotic,  
32 causing yellowish and flaccid leaves and premature fall. On the other hand, once started in  
33 the aerial part, it is characterized by the presence of yellowish branches in a very vigorous  
34 plant [6]. With the evolution of the disease is observed drying in the upper and lower part of  
35 the plant [6-8]. According to Pandey *et al.* [9], production losses due to plant diseases are a  
36 considerable challenge for the current agricultural production system worldwide,  
37 representing at least 25% of the total. Although fusariosis is a major disease, there is no  
38 record of efficient chemical control.

39 The methods used in the control of pathogenic isolates of *Fusarium* include the use of  
40 resistant varieties and soil disinfection with the chemical fungicide and crop rotation using  
41 non-host plants [10]. The use of resistant cultivars would be an alternative, but difficulties  
42 such as the identification of resistance genes or the pathogen's ability to adapt to new  
43 genotypes may make resistance a temporary solution [11]. In addition, the chemical control  
44 of *Fusarium* spp. is not fully efficient since the pathogen penetrates the vascular tissue of the  
45 plant [12]. Crop rotation would be of little efficiency since this pathogen is a soil fungus,  
46 capable of surviving for long periods in crop debris and presents several plant species as  
47 hosts [11]. Conventional synthetic fungicides are largely considered the most effective and  
48 economical means for treating the disease. However, the intensity of use and specific mode  
49 of action of most synthetic fungicides ultimately lead to resistance problems and an  
50 increased environmental cost [9, 13, 14]. Thus, alternative forms of control are of increasing  
51 interest, leading to the investigation and development of effective and sustainable products  
52 for the control of plant pathogens [15]. Zambolim *et al.* [16] reported that some micro and  
53 macronutrients have been identified as one of the main mineral elements associated with the  
54 induction of disease resistance in plants.

55 Due to their low cost, protective activity and reduced risk of resistance development  
56 controlled by the broad mode of action against pathogens, Cu compounds have been  
57 exploited to protect crops from many pests, including those that cause numerous bacterial  
58 and fungal infections [17]. According to Zambolim *et al.* [18], Cu ions in contact with spores  
59 or the pathogen's germ tube may accumulate in the membrane or penetrate and concentrate  
60 inside of the spores or mycelium, where they act by inhibiting enzymes essential to the  
61 metabolic process of microorganisms. Once accumulated in the cells, their effects become  
62 irreversible.

63 Interest in research on zinc (Zn) derivatives is increasing [19, 20], due to its strong  
64 antimicrobial activity at low concentrations and its non-toxic characteristics in adequate  
65 quantities. Zn as an essential micronutrient plays an important role in many integral  
66 metabolic processes [21]. It can also help increase chlorophyll and carotenoid biosynthesis  
67 and improve plant photosynthetic apparatus [22]. Significant optoelectric, physical and  
68 antimicrobial properties of Zn offer great potential for increasing agricultural productivity [23].  
69 Its mode of action is not completely understood, but it is known to act directly on the  
70 pathogen [18].

71 Among macronutrients of great importance to the plant, potassium (K) is one of the elements  
72 that has very positive results in reducing the incidence of pests and diseases [24, 25], being  
73 able to reduce the severity of more of 100 fungi [26]. Taiz and Zeiger [27] reported that K is  
74 an essential plant nutrient required as a cofactor for over 40 enzymes, many of which are  
75 involved in respiration and photosynthesis. As such, it is an important nutrient in plant  
76 disease prevention as it is involved in many cellular processes that influence disease  
77 severity. Its effect on the prevention of diseases caused by bacteria, fungi and nematodes  
78 has been reported [18, 28]. Increased resistance to disease from K fertilization has been

79 attributed to several mechanisms, such as cell permeability and decreased susceptibility of  
80 tissues to pathogen maceration and penetration [18, 29]. K influences the reduction of plant  
81 diseases due to the activation of enzymes involved in respiration and photosynthesis, carbon  
82 chain supply processes for defense substance synthesis, as well as stomatal regulation  
83 influencing mass flow solute transport [30].

84 The use of nutrients with antifungal action may be a strategy for controlling pathogens that  
85 cause invaluable economic losses. Nutrients such as Cu, Zn and K are easily accessible,  
86 inexpensive and still contribute to plant nutrition. Thus, the objective of this study was to  
87 evaluate the antifungal activity of Cu, Zn and K compounds against *Fusarium solani* f. sp.  
88 *piperis* growth.

## 90 **2. MATERIALS AND METHODS**

### 92 **2.1. Microorganism and cultivation**

94 The isolate of *Fusarium solani* f. sp. *piperis* CML 2466, from the Coleção Micológica de  
95 Lavras, Federal University of Lavras - MG was used. The fungus was maintained on Petri  
96 dishes containing PDA (Potato Dextrose Agar) at 4 ° C. For inoculation, 7 mm pure culture  
97 discs were transferred to Petri dishes containing the same medium. The plates were  
98 incubated at 25° C in BOD (Biochemical Oxygen Demand), photoperiod of 12h for 15 days.

### 100 **2.2. Copper, zinc and potassium concentrations**

102 Micronutrients were supplied in PDA medium as copper sulfate (CuSO<sub>4</sub>) and zinc sulfate  
103 (ZnSO<sub>4</sub>) at concentrations of 1, 5, 10, 15 and 20 mmol/L. Potassium macronutrient (K) was  
104 supplied as potassium chloride (KCl) at concentrations of 30, 60, 90,120 and 150 mmol/L.  
105 The nutrients were diluted in sterile distilled water and at the time of plating were added to  
106 the PDA culture medium in laminar flow hood. After solidification, a 7 mm diameter fungal  
107 mycelium disc with 15-day-old was transferred to the center of **each Petri dish** (68 mm  
108 diameter). The PDA medium with the fungus disc was used as control. The plates were  
109 sealed with Parafilm and maintained in BOD at 25°C with 12 h photoperiod.

### 111 **2.3. Mycelial growth**

113 The evaluation of *F. solani* mycelial growth in the control plates and treatments was  
114 determined every 2 days by measuring the diameter of the colonies in orthogonal directions  
115 with the digital pachymeter until the control treatment colony reached edge of the plate, 12  
116 days after inoculation (DAI). The growth inhibition percentage was calculated according to  
117 Guo et al. [31], where the antifungal index (%) = (1-Da / Db) x 100, where: Da gives the  
118 diameter of the growth zone in the test plate and Db the diameter of the growth zone in the  
119 control plate.

### 120 **2.4. Spore count**

122 The spore suspension was prepared by adding 20 mL of sterile distilled water to each plate  
123 containing the fungus, which was scraped with a Drigalsky handle for efficient spore  
124 extraction. The spore count was performed in a Neubauer Chamber and the suspension was  
125 adjusted to a concentration of **10<sup>6</sup>** spores/mL<sup>-1</sup>.

### 126 **2.5. Statistical analysis**

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128 The experiment was performed using a completely randomized design with 6 treatments and  
129 10 replications for each treatment (Cu, Zn and K). Each repetition consisted of a petri dish.  
130 Mycelial growth data were analyzed by linear regression. The values obtained in spore  
131 production and colony growth were submitted to analysis of variance by the F test and the  
132 means compared by the Tukey test at 5% of probability using Genes software [32].

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### 134 3. RESULTS

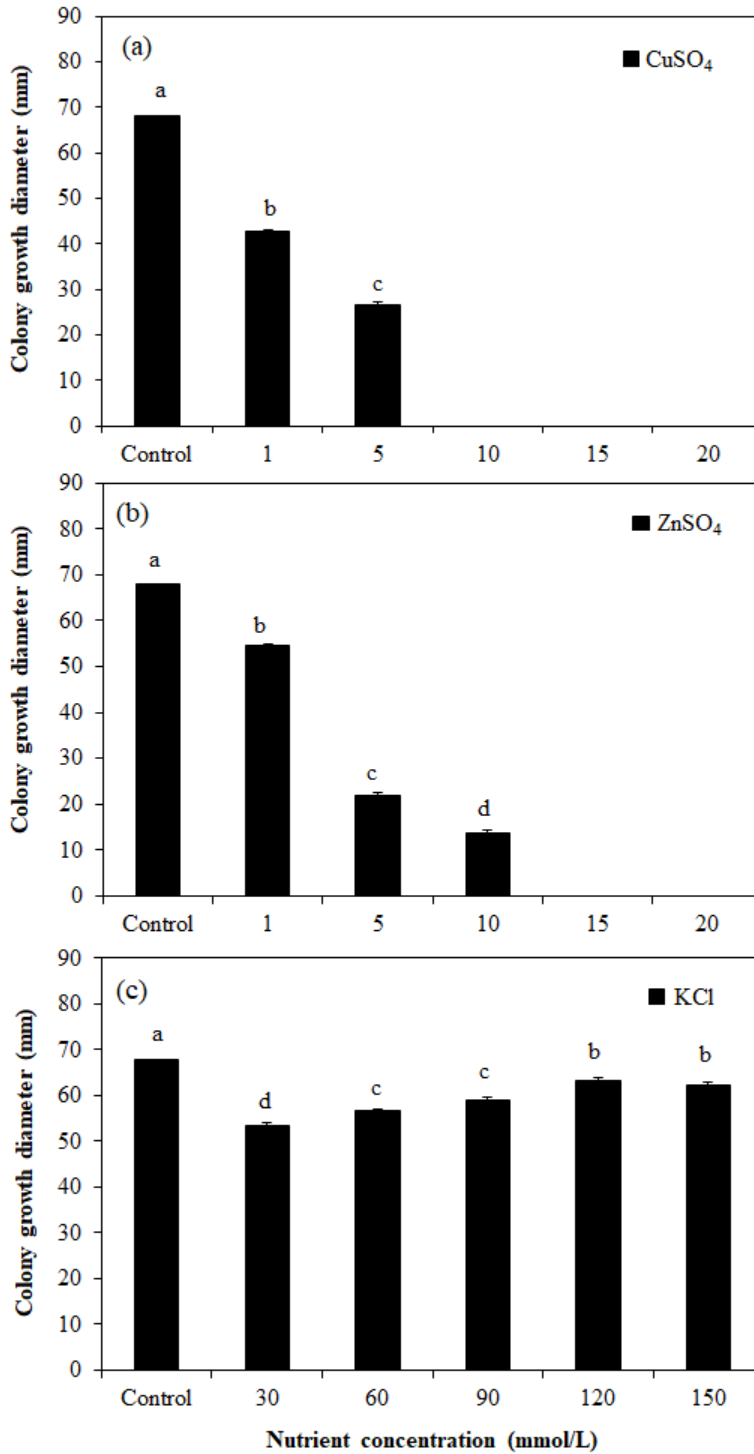
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#### 136 3.1. Mycelial growth

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138 The mycelial growth of *F. solani* was dependent on the nutrient and the dose used. Fungal  
139 growth was completely inhibited in some treatments. The  $\text{CuSO}_4$  showed fungicidal effect at  
140 concentrations of 10, 15 and 20 mmol/L, completely inhibiting growth of the colonies (Fig.  
141 1a). However, at a concentration of 5 mmol/L, a fungistatic effect was observed since  
142 mycelial growth was initiated 6 DAI (Fig. 2a), despite the projection of the line. For  $\text{ZnSO}_4$   
143 mycelial growth was completely inhibited at concentrations of 15 and 20 mmol/L, showing  
144 fungicidal effect and significantly reduced ( $P \leq 0.05$ ) at concentrations of 5 and 10 mmol/L,  
145 exerting fungistatic effect (Fig. 1b). This result is ratified after eight days of incubation by  
146 observing mycelial growth at a concentration of 10 mmol/L (Fig. 2b). There was a significant  
147 difference for KCl treatment ( $P \leq 0.05$ ) between the tested concentrations. However, there  
148 was no inhibition or reduction of fungal growth in the presence of this nutrient (Fig. 1c and  
149 2c).

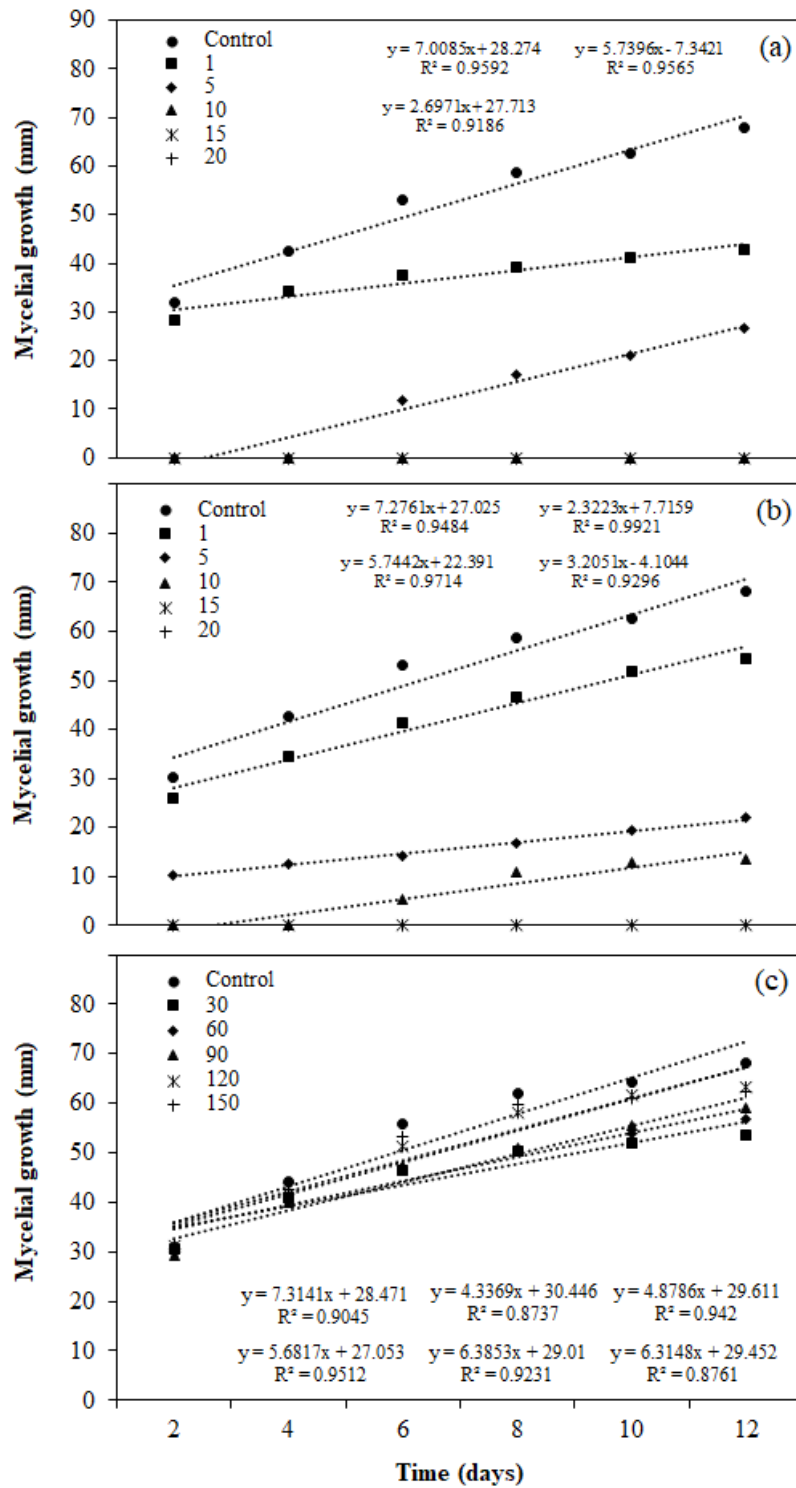
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**Fig. 1. Antifungal activities of CuSO<sub>4</sub> (a), ZnSO<sub>4</sub> (b) and KCl (c) against *F. solani* f. sp. *piperis* on PDA at different concentrations.**

Data are shown as average values. Columns followed by the identical letter are not statistically different according to by Tukey test, at 5% probability ( $P \leq 0.05$ ). Bars represent the standard error of the mean.



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**Fig. 2.** Effect of CuSO<sub>4</sub> (a), ZnSO<sub>4</sub> (b) and KCl (c) on the mycelial growth of *F. solani* f. sp. *piperis*, 12 days after inoculation. Control: only PDA medium.

\*Signifivative at 5% probabily by F test.

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### 3.2. Percent growth inhibition (P.I.).

The mycelial growth inhibition index confirmed the efficiency of the antifungal activity of CuSO<sub>4</sub> and ZnSO<sub>4</sub> (Table 1). For CuSO<sub>4</sub>, at a concentration of 5 mmol/L, there was inhibition greater than 50% 12 DAI. The other concentrations inhibited 100% fungal growth. Similar results were observed for ZnSO<sub>4</sub> (Table 1). However, for KCl, in none of the evaluated concentrations was observed P.I. below 50%. At 2 DAI there was growth induction (Table 1) with no fungistatic or fungicidal effect for this nutrient.

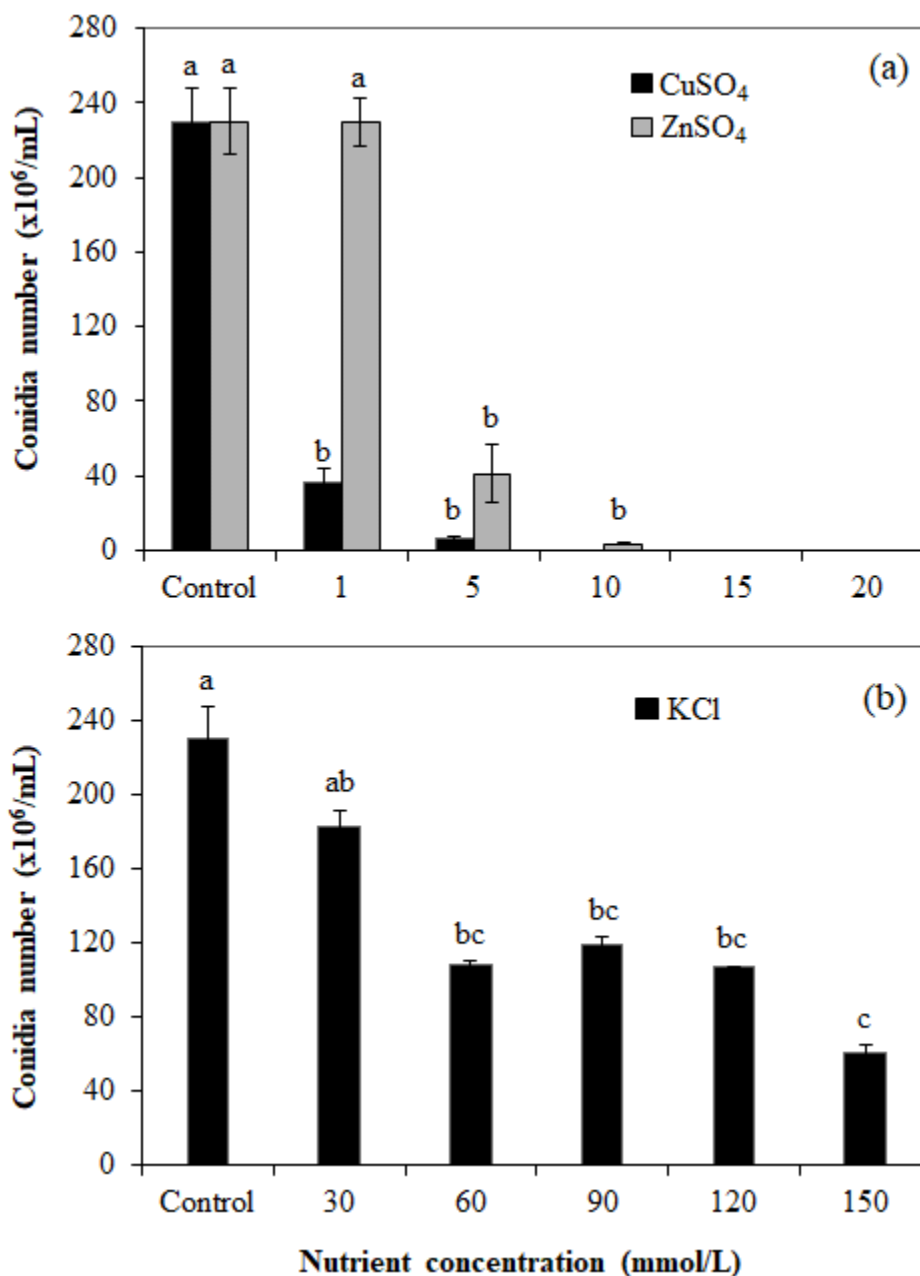
**Table 1. Percent growth inhibition (P.I.) of *F. solani* f. sp. *piperis* in CuSO<sub>4</sub>, ZnSO<sub>4</sub> and KCl**

Time (days)	P.I. (%)					
	2	4	6	8	10	12
<b>CuSO<sub>4</sub> mmol/L</b>						
Control	0	0	0	0	0	0
1	12.0	19.4	29.4	33.0	34.5	37.2
5	100	100	77.8	70.7	66.4	61.0
10	100	100	100	100	100	100
15	100	100	100	100	100	100
20	100	100	100	100	100	100
<b>ZnSO<sub>4</sub> mmol/L</b>						
Control	0	0	0	0	0	0
1	14.4	18.8	22.0	20.3	17.0	19.8
5	65.7	70.4	73.6	71.5	68.9	67.7
10	100	100	89.9	81.5	79.5	79.9
15	100	100	100	100	100	100
20	100	100	100	100	100	100
<b>KCl mmol/L</b>						
Control	0	0	0	0	0	0
30	-0.9	7.1	16.6	18.7	19.2	21.4
60	-3.8	8.0	15.6	18.8	16.2	16.5
90	2.8	9.8	15.8	17.4	14.0	13.3
120	-3.0	2.7	8.2	6.1	4.4	7.0
150	-1.4	3.7	4.8	3.8	5.0	8.3

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### 3.3. Conidia Number.

Twelve days after inoculation (12 DAI), the conidia number of *F. solani* was inhibited in the presence of CuSO<sub>4</sub>, ZnSO<sub>4</sub> and KCl. The Cu micronutrient reduced by 84% the conidial germination at 1mmol/L concentration in relation to the control. The same was not observed for Zn at the same concentration (Figure 3a, Table 2). In the other Cu and Zn concentrations, conidial germination was significantly inhibited ( $P \leq 0.05$ ), with values greater than 80%. For K treatment, there was a 20.6% reduction in the number of conidia at 30 mmol/L. The other concentrations presented a reduction greater than 50% when compared to the control (Figure 3b, Table 2).



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**Fig. 3. Conidia production of *F. solani* f. sp. *piperis* on CuSO<sub>4</sub>, ZnSO<sub>4</sub> (a) and KCl (b) in different concentrations.**

Data are shown as average values. Columns followed by the identical letter are not statistically different according to by Tukey test, at 5% probability ( $P \leq 0.05$ ). Bars represent the standard error of the mean.

**Table 2. Percent growth inhibition (P.I.) of *F. solani* f. sp. *piperis* in CuSO<sub>4</sub>, ZnSO<sub>4</sub> and KCl**



Reduction in Conidia number (%)	
mmol/L	CuSO <sub>4</sub>
1	84.2
5	97.2
10	100.0
15	100.0
20	100.0
ZnSO <sub>4</sub>	
1	0.0
5	82.2
10	98.4
15	100.0
20	100.0
KCl	
30	20.6
60	52.9
90	48.3
120	53.6
150	73.5

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#### 4. DISCUSSION

205 The widespread and persistent nature of *Fusarium* spp. may be due to its ability to maintain  
206 and multiply in a wide variety of complex carbohydrates and proteins, thus resisting adverse  
207 climates and high levels of toxic substances such as many antibiotics and fungicides. *F.*  
208 *solani* seems to incorporate some of the most difficult members of the genus [33].

209 Inhibition of mycelial growth of *F. solani* f. sp. *piperis* *in vitro* revealed significant variations in  
210 fungus sensitivity to different nutrients tested. In the present study, CuSO<sub>4</sub>, ZnSO<sub>4</sub> were  
211 more effective in inhibiting fungal growth, while KCl showed relatively weak effect comparing  
212 it to other nutrients. However, it was observed that Cu was the most effective compound  
213 against mycelial growth and *F. solani* conidia germination, showing significant inhibition at  
214 relatively low concentrations of the compound.

215 According to Resende *et al.* [34] and Melo *et al.* [35], Cu, Zn and K compounds are used for  
216 the control of microorganisms. They can have a direct effect on the pathogen (fungicidal or  
217 fungistatic effect) or activate the natural defense of plants, resulting in induced resistance  
218 [36, 37].

219 The Cu is currently used due to its antifungal properties. In particular, Cu is responsible for  
220 interference with homeostatic processes and cell membrane functions, protein synthesis  
221 damage, reactive oxygen species production, and DNA disruption [38, 39]. Civardi *et al.* [40]  
222 observed that Cu exerted toxic effect on *Rhodonina placenta* fungal cell by breakdown of  
223 different basic metabolic processes. Significant antifungal activity of Cu has been revealed in  
224 a number of pathogenic species including *Fusarium* sp., *Aspergillus niger*, *Rhizoctonia*  
225 *solani*, *Alternaria solani*, *Alternaria alternata* and *Phoma destructiva* [9, 41, 42].

226 Regarding Zn, several studies have shown its antibacterial activity [43-46]. However, there  
227 are few studies reporting the suggested mechanism for antifungal activity of Zn compounds  
228 [47, 48]. Some authors suggest that such a mechanism may be based on the formation of  
229 reactive oxygen species that disrupt the integrity of the cell membrane, preventing pathogen  
230 growth [49, 50, 46, 48]. According to He *et al.* [48], Król *et al.* [51], and Ashajyothi *et al.* [52],  
231 Zn compounds showed fungistatic potential against *Fusarium* sp., *Botrytis cinerea*,  
232 *Penicillium expansum*, *Aspergillus niger* and *Rhizopus stolonifer*. Chand *et al.* [53] observed  
233 that among the micronutrients tested Zn presented the greatest inhibition of mycelial growth  
234 of *Fusarium oxysporum* f. sp. *cuban*.

235 The marked toxic effect of Cu and Zn against fungal spores compared to mycelial growth  
236 can be attributed to the structural differences between the spore wall and the fungal  
237 vegetative phase. Bartnicki-Garcia [54] observed that the chitin content of many fungal  
238 species is significantly higher in the hyphae wall compared to the spore wall, making the  
239 latter more susceptible to some compounds. In addition, during the spore germination  
240 process, the presence of enzymes such as disulfide reductase and glucanases result in  
241 weakening of the cell wall, facilitating germ tube lengthening and thus creating sites of  
242 greater sensitivity to toxic substances in contact with the cell fungal [54]. In general, conidial  
243 germination may reflect reproductive capacity and fungal development. Savi *et al.* [20]  
244 suggest that the effect of Zn compounds on fungal growth may be related to their property,  
245 altering reproductive capacity in terms of conidia viability. Malandrakis *et al.* [55], studying  
246 the effect of copper and zinc on various microorganisms, found that Cu was effective against  
247 *Alternaria alternata*, *Botrytis cinerea*, *Monilia fructicola*, *Verticillium dahliae*, *Colletotrichum*  
248 *gloeosporioides*, *Fusarium oxysporum* f. sp. *radicis lycopersici* and *Fusarium solani* while Zn  
249 exerted a fungicidal effect against *M. fructicola*, *F. solani* and *V. dahliae*.

250 Although K has not exerted inhibition or reduction of *F. solani* mycelial growth and spore  
251 production, there are reports in the literature that K acts as an inducer of resistance to plant  
252 diseases [56-58]. The use of K as a plant fertilizer may decrease the incidence of fungal and  
253 bacterial as well as insect diseases [59], mainly due to changes in primary metabolism and  
254 plant hormonal responses [29, 57]. Dordas [60] observed that the application of KCl on  
255 foliage can prevent the attack of mildew on wheat.

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## 257 **5. CONCLUSION**

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259 The results obtained in this work provide evidence that copper and zinc exhibited antifungal  
260 activity against *F. solani* f. sp. *piperis* under laboratory conditions. High antifungal activity  
261 was observed at low concentrations of copper (10 mmol/L) and zinc (15 mmol/L), favoring  
262 the use of these compounds to control mycelial growth and conidia production. On the other  
263 hand, no potassium concentration was active against the fungus. Based on these results,  
264 field experiments can be performed to verify the performance of these nutrients as *Fusarium*  
265 growth inhibitors, as well as the physiological and nutritional behavior of the plant.

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## 268 **COMPETING INTERESTS**

269

270 The authors declare that they have no conflicts of interest.

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## **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

Here is the Definitions section. This is an optional section.

**Term:** Definition for the term