



Behavior of *Dioscorea alata* Slices Treated with Aqueous Extracts of *Ocimum gratissimum* and *Chromolaena odorata* before Inoculation by *Colletotrichum* sp. in Daloa, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Yam, *Dioscorea* spp. (L) is an important foodstuff that plays a key role in the agricultural system in Côte d'Ivoire. This plant is however subject to several diseases during its cultivation and conservation. The losses caused by rots of tubers in storage constitute a major risk for economic profitability and for the food safety of consumers. The development of an effective and environmental friendly control method has been initiated. The objective of this work is to improve the conservation of *Discorea alata* tubers through the use of aqueous extracts of *Ocimum gratissimum* and *Chromolaena odorata*. To do this, three different doses (33 g/l; 39 g/l and 50 g/l) of aqueous extracts of *Ocimum gratissimum* and *Chromolaena odorata* were applied to yam slices before inoculation with *Colletotrichum* sp. Results showed that applying different doses of aqueous plant extract to yam slices before inoculation of the fungal strain caused less rot. The aqueous extracts of the two plants showed antifungal activity against *Colletotrichum* sp. This antifungal activity was more effective with the 50 g/l dose of *Ocimum gratissimum* compared to the extract of *Chromolaena odorata*.

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1. INTRODUCTION

Yam, *Dioscorea* spp (L) is a major food plant in many tropical countries of Asia, South America and Africa [1]. Yam cultivation contributes to the food security of over 300 million people [2]. In West Africa, yam plays an important role economically and nutritionally and also in socio-cultural rites [3]. Côte d'Ivoire is the third largest producer of yams in the world after Nigeria and Ghana. With an annual production of 6 million tonnes per year, yam represents the first food crop and the most important staple food in Côte d'Ivoire [3]. Yam is consumed by more than two-thirds of the population. It is the main food and has a very important socio-cultural dimension during the yam festivals of some peoples [4][5]. The fresh tuber is cut, dried and then transformed into flour to make fritters or couscous dishes [6]. Yam is a high energy food, low in fat and the richest of all tubers in protein. It consists of 50 to 80% water, 32% carbohydrates, the main constituent of which is starch, 5% protein [7].

Unfortunately, many ecological and parasitic constraints limit yam production [8][5]. Indeed, the most important losses are observed during storage and limit the food intake of populations in many developing countries [8]. They are caused by external agents such as insects, rodents and molds. In addition, the high-water content of tubers, associated with the injuries they sustain during and after harvest, expose them to the microorganisms that cause them to rot [9]. Thus, according to [10], post-harvest rots in yam tubers are estimated at around 20 to 30% in Côte d'Ivoire. Indeed, the work of [9] and [11] showed that *Penicillium* sp., *Aspergillus* sp., *Botryodiplodia* sp., *Mucor* sp. and *Colletotrichum* sp. are among others the fungal agents responsible for these rots during storage. To control the fungi responsible for rotting yam tubers, different types of treatments have been used. These are fungicides such as benomyl, captan and thiabendazole [11].

However, these chemicals have many disadvantages for people and biodiversity. Thus, biological control using plant extracts is a promising alternative that can be used in post-harvest [12]. The objective of this study is to evaluate the behavior of *D. alata* slices treated with aqueous extracts of *Ocimum gratissimum* and *Chromolaena odorata* then inoculated with the strain.

2. MATERIALS AND METHODS

2.1 Study Area

This experiment was carried out in 2019-2020 in the agricultural improvement and production laboratory of the University Jean Lorougnon Guédé in the Haut Sassandra region.

The town of Daloa (6°53 north latitude and 6°27 west longitude) is the capital of the Haut-Sassandra region and is located about 141 km from Yamoussoukro and about 400 km from Abidjan [13]. This region has an area of 15.200 km² for an estimated population of 1,430,960 inhabitants. The soils of the region are predominantly ferralitic and are generally very deep with a high level of organic matter [14].

2.2 Materials

2.2.1 Plant and fungal material

The plant material used consisted of tuber of *Dioscorea alata* variety bête-bête from the previous harvest (Fig.1A) cut into slices (Fig.1B) and leaves of *Chromolaena odorata* (Fig.1C) and *Ocimum gratissimum* (Fig.1D).

The fungal material is a pre-existing strain of *Colletotrichum* sp. Isolated from infected *Arachis hypogaea* leaves (Fig.2).

2.3 Methods

2.3.1 Sampling

Apparently healthy *D. alata* yam tubers were collected in "Digba", a village 15 km from Daloa town. The leaves of *C. odorata* and *O. gratissimum* were collected at the University Jean Lorougnon Guédé. The yam tubers and the leaves of the two plants were then put in a bag and then transported to the laboratory for the conduct of the experiment.

2.3.2 Preparation of the culture medium and purification of the pre-existing strain of *Colletotrichum* sp.

PDA medium was used for the purification of the pre-existing strain of *Colletotrichum* sp. For 1 liter of PDA medium, 200 g of potato was diced and boiled for 1 hour. The filtrate was collected in an Erlenmeyer flask, to which 20 g of glucose and

20 g of Agar-agar were added. The mixture was adjusted to 1 liter of sterile distilled water and autoclaved at 121 °C for 30 minutes under a pressure of 1 bar. When the medium is supercooled, 0.5 g of antibiotic (Amoxicillin) was added to it to prevent the proliferation of saprophytic bacteria which interferes with the

development of the fungal strain [15]. After cooling, the medium was poured into petri dishes 9 cm in diameter under a laminar air flow hood, in the presence of a flame. The fungal strain was subcultured onto the solidified PDA media contained in the petri dishes for purification.



Fig. 1. Different types of plant material, A: *D. alata* tuber; B : *D. alata* slice; C : *C. odorata* fresh leaves; D : *O. gratissimum* leaves

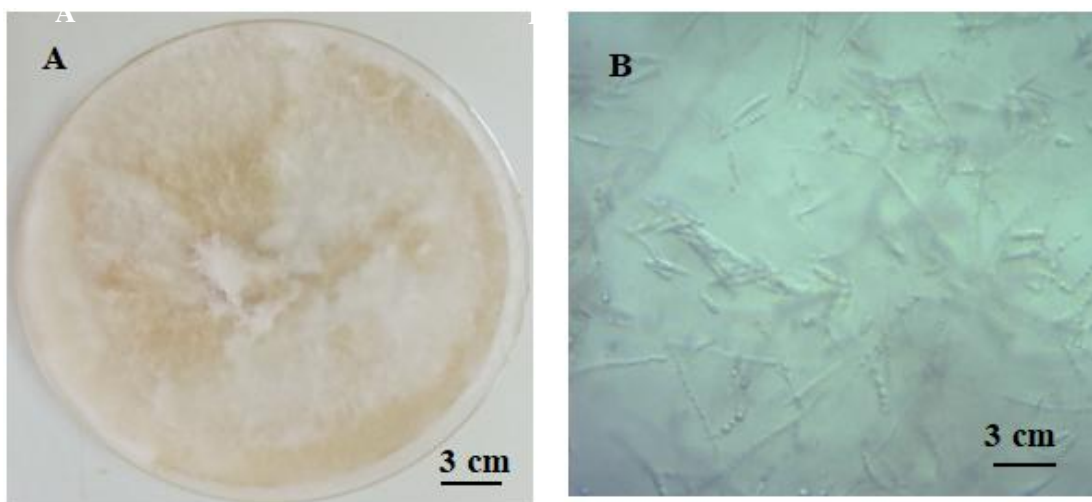


Fig.2. Strain of *Colletotrichum* sp. A: Pure culture of *Colletotrichum* sp, B: Conidia of *Colletotrichum* sp.

2.3.3 Preparation of different plant extracts

The fresh leaves of *C. odorata* and *O. gratissimum* were collected at Jean Lorougnon Guédé University and rinsed with distilled water and then dried in the shade in the laboratory at room temperature (25 °C) for two weeks. The dried leaves of each plant were crushed and weighed in three different amounts of 33 g, 39 g and 50 g, based on the preliminary results obtained in the literature. These quantities were each infused in 1 l of water for 24 h to obtain the mass concentrations of 33 g/l, 39 g/l and 50 g/l. The infusions obtained were filtered using filter paper according to the method of [16] and each concentration of extract was used to test the behavior of *Colletotrichum* sp on *D. alata* slices.

2.3.4 Preparation of *Dioscorea. alata* slices

Apparently healthy *D. alata* tubers were washed with distilled water and disinfected with 70% alcohol for 3 min. After disinfection, these tubers were cut into slices 3 cm thick and 9 cm in diameter. Eighty slices were used to perform the inoculation test. For each type of extract, 40 slices were used, i.e. 10 slices per dose (33; 39 and 50 g/l) of extract and 10 control slice. Two perpendicular lines were drawn on one of the faces of the previously disinfected slices. Then a 1 cm deep hole was made in the center of each slice at the intersection of the two lines using a 0.2 cm diameter cookie cutter (Fig.3). This hole served to introduce the fungal inoculum.

2.3.5 Spraying, inoculating and incubating the slices

The yam rings were sprayed with different doses of plant extracts. For this purpose, forty rings divided into ten with 5 ml of each dose of extract (33 g/l, 39 g/l and 50 g/l) of the two plants. Two days after spraying, a fragment of *Colletotrichum* sp. 0.2 cm in diameter and 1 cm in length was introduced into the hole already made in the center of the slices [17] while orienting the face of the inoculum carrying the mycelium downwards. The ten control discs were inoculated with 0.2 cm in diameter of sterile PDA medium and sprayed with 5 ml of sterile distilled water also two days later.

The inoculated washers were then stored in sterile transparent polyethylene bags. These sachets contained blotting paper soaked in sterile distilled water in order to maintain a high relative humidity and avoid any other external contamination.

The sachets containing the slices were incubated in the laboratory at 25 °C. The different doses of extract were used to spray the slices every 2 days for 10 days.

2.3.6 Observation and evaluation of the parameters on the discs of *Dioscorea alata* growth of the fungal strain

Daily observations were made for ten days after inoculation with the fungal strain. During this period, the presence (score 1) or absence (score 0) of mycelium as well as rots on the yam rings, caused by *Colletotrichum* sp., depending on the dose and the type of extract sprayed were observed.

2.3.7 Calculation of rots diameters

Measurements of the change in surface rot diameters were carried out daily depending on the dose and the type of extract. These measurements were made using a ruler graduated in cm along the two perpendicular lines (x and y) drawn on the yam slices. The average of the measurements for each slice was obtained according to the following formula:

$$X_i = \frac{A + B}{2} \quad (1)$$

X_i : average of the decay measurements in cm for a slice of yam;

A: diameter of rots along the x axis in cm;

B: diameter of rots along the y axis in cm.

2.3.8 Heights calculation and rot volume

The different heights of rot of the rings were measured on the tenth day as a function of the different doses of extracts from the two plants. For this purpose, the washers were split into two equal parts passing through the center with a sterile knife and the rot heights were measured with a ruler. The measurements of the different heights of decay were used to calculate the volume of decay caused by the fungal strain for each disc according to the formula of [18].

$$V = \pi r^2 x h \quad (2)$$

V: rot volume in cm³

π : 3.14

r²: rot radius of each washer in cm²

h: rot height of each slice in cm

2.4 Statistical Analysis

A one-way analysis of variance (ANOVA 1) was performed at threshold $\alpha = 0.05$. Statistica 7.1 software was used to assess the type and dose effect of extract on the diameter, length, height and rot volume of each slice. If there was a significant difference between the means for each parameter, they were classified using Fisher's LSD test.

3. RESULTS

3.1 Presence of Mycelium According to the Different Doses of Aqueous Extract

The fungal strain inoculated into *D. alata* slices following treatment with the different doses of the two types of extracts was able to grow. This growth is materialized by the presence of mycelium on the surface of the slices. These mycelia evolved slowly as the doses of each plant extract increased until they disappeared from the surface of the *D. alata* rings at the end of the experiment (Fig.4). For this purpose, analysis of the data showed a significant difference ($P = 0.000$). Means were low and ranged from 0% to 48% with two homogeneity groups. The first group with the strong presence

of mycelium (48 % and 40 %) was obtained respectively with the doses 33 g/l and 39 g/l of the extract of *C. odorata*. The weak presence of mycelium (8 %; 4 % and 0 %) are from the second group and were obtained with the respective doses of *C. odorata* at 50 g/l and *O. gratissimum* at 33 g/l; 39 g/l and 50 g/l. These latter doses prevented the mycelium from growing on the yam rings (Table 1).

3.2 Evolution of Porrage Diameters According to the Different doses of Aqueous Extracts

The mean rot diameters ranged from 0.00 to 0.37 cm at the two extracts. The analysis showed that the different doses of the two extracts had a significant effect ($P = 0.000$) on the pore diameters of the *D. alata* discs treated before inoculation. Classification of the porosity averages revealed two groups of homogeneity. The first with the strong rots (0.37 and 0.30 cm) was obtained with the doses of *C. odorata* respectively at 33 g/l and 39 g/l. The second group with the weak rots (0.04; 0.02 and 0.00) was obtained respectively with the doses 50 g/l of *C. odorata* and 33 g/l, 39 g/l, 50 g/l of *O. gratissimum* as well as the witness. However, the dose of *O. gratissimum* 50 g / l was the most effective on mycelial diameters (Table 1).

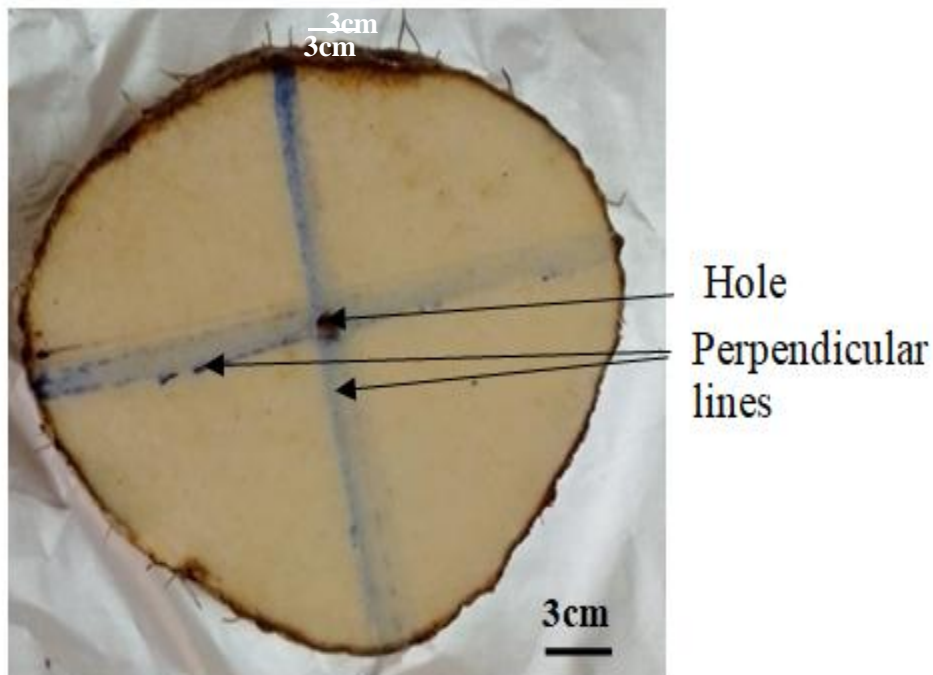


Fig. 3. Slice used for inoculation



Fig. 4. slice sprayed with the doses aqueous extracts then inoculated with the fungal strain

Table 1. Presence of mycelium and rot diameter on the inoculated slices after intake of doses of extracts

Dose of extract (g/l)	Presence of myceliums (%)	Rot diameters (cm)
Ct	50 ± 0.32 a	0.46 ± 0.50 b
<i>C. odorata</i> 33	48 ± 0.50 a	0.37 ± 0.48 a
<i>C. odorata</i> 39	40 ± 0.50 a	0.30 ± 0.45 a
<i>C. odorata</i> 50	8 ± 0.27 b	0.04 ± 0.17 b
<i>O. gratissimum</i> 33	8 ± 0.27 b	0.04 ± 0.13 b
<i>O. gratissimum</i> 39	4 ± 0.20 b	0.02 ± 0.10 b
<i>O. gratissimum</i> 50	0 ± 0.00 b	0.00 ± 0.00 b
F	8.91	8.32
P	0.000	0.000

Values followed by the same letter in the same column are statistically equal to the threshold; $\alpha = 0.05$. F: Fischer value, P: Probability. Ct: Control, *C. odorata* 33: *Chromolaena odorata* at 33 g / l; *C. odorata* 39: *Chromolaena odorata* at 39 g / l; *C. odorata* 50: *Chromolaena odorata* at 50 g / l; *O. gratissimum* 33: *Ocimum gratissimum* at 33 g / l; *O. gratissimum* 39: *Ocimum gratissimum* at 39 g / l; *O. gratissimum* 50: *Ocimum gratissimum* at 50 g / l.

Table 2. Rot height and volume of inoculated slices after intake of doses of extracts

Dose of extract (g/l)	Rot height (cm)	Rot volume (cm ³)
Ct	1.70 ± 0.26 a	140.32 ± 92.12 a
<i>C. odorata</i> 33	2.16 ± 0.45 a	179.46 ± 70.88 a
<i>C. odorata</i> 39	3.56 ± 0.53 a	89.16 ± 69.17 a
<i>C. odorata</i> 50	2.24 ± 0.35 a	100.84 ± 61.38 a
<i>O. gratissimum</i> 33	3.14 ± 0.43 a	70.17 ± 85.53 a
<i>O. gratissimum</i> 39	1.66 ± 0.32 a	152.95 ± 94.11 a
<i>O. gratissimum</i> 50	2.38 ± 0.36 a	107.22 ± 108.56 a
F	1.56	0.95
P	0.22	0.47

Values followed by the same letter in the same column are statistically equal to the threshold; $\alpha = 0.05$. F: Fischer value, P: Probability. Ct: Control, *C. odorata* 33: *Chromolaena odorata* at 33 g / l; *C. odorata* 39: *Chromolaena odorata* at 39 g / l; *C. odorata* 50: *Chromolaena odorata* at 50 g / l; *O. gratissimum* 33: *Ocimum gratissimum* at 33 g / l; *O. gratissimum* 39: *Ocimum gratissimum* at 39 g / l; *O. gratissimum* 50: *Ocimum gratissimum* at 50 g / l.

3.3 Evolution of the Height and Rotting Volume of the Washers According to the Different Doses of Aqueous Extracts

The sliced yam rings after ten days of observation showed that the rots caused by the fungal strain had developed in depth (Fig.5). The height of the rot varied from dose to dose of plant extract. However, statistical analysis showed that there is no significant difference between rot heights ($P = 0.22$; $F = 1.54$) and decay volumes ($P = 0.47$; $F = 0.95$) of *D. alata* slices regardless of the dose and type of *C. odorata* and *O. gratissimum*. The heights and volumes of decay are recorded respectively in Table 2.

4. DISCUSSION

The behavior of *Dioscorea alata* slices treated with aqueous extracts of *Ocimum gratissimum* and *Chromolaena odorata* before inoculation with *Colletotrichum* sp. was studied. The results showed low sporulation of the mycelia of *Colletotrichum* sp. on yam rings treated with the doses of aqueous extracts of *C. odorata* and *O. gratissimum*. The rot diameters varied depending on the dose and type of extract. Regarding the heights and volumes of rots, there was no difference.

The sporulation of the mycelia of *Colletotrichum* sp. on yam rings treated with the doses of aqueous extracts of *C. odorata* and *O. gratissimum* was low. This is because fungal

sporulation decreased as the extract doses increased. This decrease in sporulation was enhanced with doses of *O. gratissimum* extract. This could be due to its phytochemical composition of *O. gratissimum* extract which could have a fungicidal effect on the fungal strain. These observations align with those of [19] who claimed that the number of fungi colonies decreased as the concentrations increased. According to them, the essential oil of *O. gratissimum* acted in a dose response relationship. Indeed, the extract of *O. gratissimum* was more effective compared to *C. odorata* extract. This strong antifungal activity of *O. gratissimum* could be due to its chemical composition different from that of *C. Odorata*. [20] has shown in his studies that plant matter in active chemical compounds of *O. gratissimum*, includes catechetal tannins, gall tannins, flavonoids, anthocyanins and steroids that have a strong activated antifungal and antibacterial. Also, the activity of *O. gratissimum* on microorganisms could be due to major compounds such as thymol, Z-terpinene and p-cymene [21] and [22]. In addition, the two extracts could have phytochemical constituents with different activities due to the mode of action of each. [23] reported that *C. odorata* extract contains antifungal substances such as sesquiterpenes, monoterpenes, flavonoids and tannins. According to these authors, these substances have shown efficacy on many fungal strains such as *Aspergillus ochraceus* and *Penicillium digitatum*.

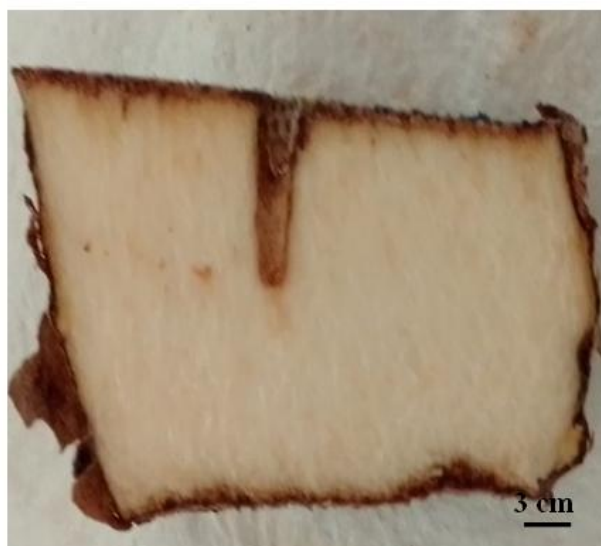


Fig. 5. Height of treated puck rot before inoculation *Colletotrichum* sp.

The presence of some mycelial fragments observed on the last day of the experiment on discs inoculated with *Colletotrichum* sp and treated with *C. odorata* could be due to a temporary lytic activity of the *C. odorata* extract. These statements corroborate the work of [23] who noted an efficacy of the extract of *C. odorata* on *Penicillium* sp. and *Aspergillus* sp. strains the first three days after treatment and the decrease of its antifungal activity the following days.

According to them, this observation could be due to the volatile nature of the main antifungal compounds of this extract, which are the monoterpenes and sesquiterpenes. Thus, the extract of *C. odorata* would have a non-persistent antifungal activity unlike the permanent antifungal activity observed with the extract of *O. gratissimum*.

The fungal strain inoculated into the yam slices previously treated with the different doses of aqueous extract of the two plants induced rots of different sizes on the surface and in depth of these. The presence of rots on the washers could be explained by the ability of the fungal strain to cause damage. These statements corroborate those of [24] and [25]. According to these authors, the genus *Colletotrichum* is generally associated with postharvest yam rots. Also, the rots on the yam rings could be due to the ability of this fungus to use the nutrients present in the yam to grow. However, this growth was short-lived. This could explain the fact that the extracts of these plants would have quickly neutralized the spores of *Colletotrichum* sp on the slices of *D. alata*. Results confirm those of [17], who showed in their works that, rots of yam tubers treated with *Trichoderma* sp. before the addition of the other fungal strains had evolved less than those of the tubers inoculated with the strains before the intake of *Trichoderma* sp.

The extract of *O. gratissimum* at 50 g/l was the most reduced the development of rots at the level of the rings than the other doses of extract, whatever the type of plant. However, [26], in their work carried out with the extract of *C. odorata* on two isolates of *Fusarium oxysporum* responsible for the fatal yellowing of banana leaves, found that from 30 g/l, the percentage inhibition remains constant and that an increase in the concentration would have no effect on the treated fungi. This difference in results with those of our work could be explained on the one hand by the difference in the plant material used and on the other hand in the experimental conditions.

5. CONCLUSION

The study was initiated to control the fungi responsible for rots of *Dioscorea alata* in stock. Results showed that *Colletotrichum* sp. inoculated to *D. alata* slices pre-treated with different doses of *C. odorata* and *O. gratissimum* extracts caused low-grade rots on the inoculated slices. Both *C. odorata* and *O. gratissimum* extracts have antifungal activity. However, *O. gratissimum* extract showed stronger antifungal activity than *C. odorata* extract. The *C. odorata* extract showed temporary inhibition of mycelium at all doses, whereas the *O. gratissimum* extract permanently inhibited mycelial growth on the inoculated slices. The 50 g/l dose of *O. gratissimum* was the most effective.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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