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ISSN: 2224-0616

Int. J. Agril. Res. Innov. Tech. 14(2): 62-73, December 2024Available online at https://ijarit.onlineDOI: https://doi.org/10.3329/ijarit.v14i2.79401https://www.banglajol.info/index.php/IJARIT

## *In-vitro* effectiveness of aqueous extract of organic compost against *Helminthosporium turcicum* (cryptogamic disease pathogen of maize)

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Received 5 October 2024, Revised 10 December 2024, Accepted 25 December 2024, Published 31 December 2024

#### ABSTRACT

Sustainable agriculture requires the application of biofertilizers and biopesticides in farming systems. The aim of the study was to analyze *in-vitro* the effect of aqueous extracts from organic compost on the development of cryptogamic disease in maize plants for yield improvement. The study was carried out in the laboratory using a pure strain of Helminthosporium turcicum previously isolated from infected maize leaves showing symptoms of the disease. Aqueous substrates of unsterilized and sterilized composts, with the concentrations 0.5, 1.0 and 2.0 mL were respectively mixed with liquid PDA at 19.5; 19 and 18 mL for a final volume of 20 ml, then aseptically poured into 90 mm diameter Petri dishes under the laminar flow hood. After solidification, 7 mm-diameter mycelial discs from young *H. turcicum* cultures were deposited on the agar pellets. 48 hours after incubation, mycelial growth was measured 4 times until the positive control Petri dish was invaded by mycelial filaments. The results showed that the mycelial growth of the H. turcicum strain in the presence of the sterilized aqueous compost extract was significantly reduced. Increasing the concentration significantly inhibited strain growth to the order of 95.89% at a concentration of 2 mL sterilized aqueous compost extract. Similarly, in the presence of the unsterilized aqueous compost extract, the mycelial growth of *H. turcicum* was also reduced. The Increasing concentration inhibited the pathogen growth by up to 76.92%, at the concentration of 2 mL. These results showed that the aqueous extract of organic compost contains compounds that could be responsible for producing suppressive substances accountable for the mechanisms inhibiting the mycelial growth of *H. turcicum*.

Keywords: Aqueous compost extract, Mycelial growth, *Helminthosporium turcicum*, PDA middle, Maize

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Cite this article as: Kutnjem, D., Ayo, A., Onguene, E.M.A., Ndemé, C.C.E., Bekolo, N., Katamssadan, T.H. and Carnot, A.C. 2024. *In-vitro* effectiveness of aqueous extract of organic compost against *Helminthosporium turcicum* (cryptogamic disease pathogen of maize). *Int. J. Agril. Res. Innov. Tech.* 14(2): 62-73. https://doi.org/10.3329/ijarit.v14i2.79401

#### Introduction

The demographic explosion combined with rapid and anarchic urbanization contributed to a significant production of waste in African cities (Kaudu and Kilewa, 2021), particularly in Cameroon cities. The management of these wastes is still challenging, both financially and environmentally. This is due to the everincreasing cost of the removal of these wastes, as well as the risk of insufficient treatment outlets in the medium term, and wastage of raw materials

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(Nkula et al., 2023). The conventional solution commonly used is to bury waste in landfill sites, creating countless contaminated sites for future generations (Sombo, 2021). Countries like Cameroon need to promote the conversion of a part of these urban wastes or biowastes to compost and its extract that will meet acceptable standards. This is because, good-quality compost provides extracts which may possess growing and protective properties which are useful in sustainable agriculture (Kutnjem et al., 2018a; Gbilimou, 2024). Indeed, growing media such as soil often have suppressive powers that enable them to reduce certain plant diseases (Kutnjem et al., 2018b). Cotxarrera et al. (2002) pointed out that these suppressive properties of compost extracts are provided either by reducing the pathogen's saprophytic growth and survival, by reducing disease expression, or by both mechanisms. However, Tian et al. (2002) revealed that the majority of soils are not suppressive and disease control is based mainly on chemical control. Nevertheless, Ozbay and Newman (2004) confirmed that the repeated use of synthetic pesticides often leads to environmental pollution, the appearance of resistant strains and an increase in the number of residues on cultivated crops. Mouria et al. (2013) reported that over the last few decades, interest in the use of biological control methods has grown considerably with increasing concern for sustainable agriculture using organic amendments such as animal manure or compost. Compost is a hygienic product of stable composition, rich in humic substances, resulting from biochemical conversion by aerobic biodegradation of organic matter (Soudi, 2005; De Corato, 2020).

Compost extracts have been widely used by farmers for their beneficial effects on plants. Over the last two decades, several studies (Yohalem et al., 1996; Reuveni et al., 2002; Al-Dahmani et al., 2003) have shown that compost extracts are effective in controlling several pathogens, both telluric and foliar. The work of Znaidi (2002) and Zaghouani (2024) has demonstrated the efficacy of compost extracts on various Fusarium genera and other telluric pathogens. This efficacy is due in part to the differences in the preparation procedures of the extract of compost, the source, the composition, the compost maturity and the time. Compost derived composting from household waste has suppressive effects on diseases caused by telluric pathogens such as Phytophthora, Pythium, Fusarium and Rhizoctonia etc., or foliar pathogens such as Cochiobolus carborum, Plasmopara viticola, Erysiphe betae, Botrytis cinerea etc. (Schönfeld et

*al.*, 2003; Hoitink *et al.*, 1997, Curadelli *et al.*, 2023). In addition, the various pathogens do not behave in the same way towards the compounds enclosed in the extracts of compost (Al-Dahmani *et al.*, 2003; Milinković *et al.*, 2019). The ability of composts to suppress plant diseases has been attributed to their chemical composition, the availability of nutrients and their microbial composition, due both to the supply of microorganisms and to the stimulation of those in the soil (Pérez-Piqueres *et al.*, 2006; González-Hernández, 2021).

The study carried out on the phytosanitary effect of household waste compost on parasitic diseases of maize at the Nkolfon in Cameroon, showed a significant reduction in the incidence and severity of helminthosporiosis and fusariosis was observed (Kutnjem *et al.*, 2018b). However, if many studies have been carried out with compost extracts on Fusariums (Pharand *et al.*, 2002; Reuveni *et al.*, 2002; Znaidi 2002; Hibar *et al.*, 2006) etc. there is little or no similar research with done against *Helminthosporium* sp. The aim of this work was therefore to study the effect of sterilized and nonsterilized aqueous extracts of household waste compost on the mycelial growth of *H. turcicum*.

#### **Materials and Methods**

#### Study areas

The study was carried out in the experimental sites of Nkolfon located on the western outskirts of the Yaoundé 3 district in the Centre region of Cameroon and the laboratory in of Phytopathology of the University of Yaounde I (Cameroon). The sites were located between latitudes 3°46'01" N and 3°76'67" N and longitudes 11°25' 60" E and 11°43' 33" with an average altitude of 726 m above sea level (Fig. 1.). The fungal material consisted of H. turcicum strains and spores were collected from maize leaf fragments grown in the experimental sites. The biological material consisted of maize (Zea mays) leaf fragments bearing the symptom of H. turcicum attack (Fig. 2.). Leaves were harvested from infected plants under natural conditions and the pathogen was purified in the laboratory. The chemical material used was Ridomil Gold plus 66WP, which is a fungicide commonly used in cocoa farms for antifungal control, with an active ingredient consisting of 60g of mefenoxam. The experimental set-up is thus a completely randomised two-factor trial in which the treatments (compost extract) represent factor A and the pathogen is part of factor B.



Fig. 1. Localization of the study areas (Nkolfon) in Yaounde city.



Fig. 2. Fragments of maize leaves showing symptoms of attack by *Helminthosporium turcicum*.

#### Preparation of the Potato Dextrose Agar (PDA) culture medium

To obtain the PDA medium, 200 g of potatoes were prepared in tap water for 30 min. The cooked apples were pressed through filter paper and the apple juice was collected in a 1000 ml beaker. 15 g agar and 15 g dextrose were added (Kutnjem *et al.*, 2018a). The mixture was made up to 1000 ml with distilled water and stirred on a magnetic stirrer. The pH was adjusted to 6.0 if necessary. The resulting solution was autoclaved at 120°C for 30 minutes and stored in the refrigerator. This medium was used to culture pure strains and for mycelial growth inhibition tests on *H. turcicum* (Kutnjem, 2020).

#### Obtaining the pure strain of Helminthosporium turcicum

The pure strain of *H*. turcicum was isolated from infected leaves showing symptoms of the disease. The leaves were washed in tap water and then disinfected using cotton wool soaked in 95% alcohol. Using a scalpel, the infected parts of the leaf (bearing the necrosis) are removed and then incubated in Petri dishes containing the PDA medium. The dish was closed, sealed with cling film and incubated in a culture chamber at 22-24°C with a 12-hour photoperiod. The mycelium develops from the explant and, after 08 days, reaches sufficient growth for purification. Purification was carried out by successively transferring an agar fragment taken from the mycelium growth front onto a PDA medium. This operation was repeated as many times as necessary until pure cultures were obtained, which were then stored in pillboxes in sterile distilled water (Nyassé, 1992; Ondo, 2006; Kutnjem, 2020). Identification was made using microscopic observations of the mycelium and an identification key (Hsieh and Goh, 1990) as shown in Figure 3.



Fig. 3. Obtaining a pure strain of *Helminthosporium turcicum* (A= pure strain; B= mycelium).

#### Koch's postulate

Healthy maize leaves were harvested very early in the morning and transported to the laboratory. They were cleaned with 70° alcohol and rinsed with sterile distilled water. They were then cut into small pieces and placed in the Petri dishes covered with cotton soaked in distilled water. This was to maintain the humidity and prevent the leaf from drying out, in order to make the leaf blade more receptive to inoculation (Nyassé, 1992; Kutniem et al., 2018b). The release of conidia forming unity (cfu) was first promoted by heat shock, by subjecting the suspension (mycelium collected from the growth front in a Petri dish and mixed with distilled water) to a temperature of 4°C in a refrigerator for 5 minutes, then to room temperature in a dark room for 30 minutes. One ml of the *H. turcicum* conidial suspension was placed in the centre of a leaf fragment using a micropipette. This fragment was incubated in a Petri dish in a dark room at a temperature between 24 and 26°C. 08 days after inoculation, the presence and development of necrosis revealed successful infection by the pathogen (Fig. 4.), confirming its identity (Nyassé, 1997; Kutnjem, 2020).



Fig. 4. Pathogenicity test on maize leaf in the Petridish.

#### Evaluation

The evaluation focused on the mycelial growth of the fungus, the colour of the fungus and the appearance of the mycelium. Mycelial growth was measured at 2, 4, 6 and 8 days after incubation. To do this, two perpendicular lines passing through the centre of the explant are drawn on the Petri dish. The straight lines will be used to measure the diameters of the mycelial colonies.

#### **Obtaining aqueous compost extract**

The compost was obtained by small-scale composting of household waste collected selectively from households and markets in the city of Yaounde. Compost juice or aqueous compost extract was prepared using the aerobic method or the original extraction method by fermentation developed by the German Heinrich Weltzeien (1992) and improved by Will Brinton on the East Coast of the United States (1996). The method involves mixing one volume of compost with two or five volumes of demineralised water (1:2 and 1:5 (v:v; compost: water)) in a polyethene (PE) bottle that facilitates daily manual agitation. The mixture was then manually shaken for two minutes, then incubated or left to ferment in the open air at room temperature. After an absorption period of 3 or 7 days, referred to as the 'extraction time', the solution was passed (filtered) through a muslin cloth. The extract obtained was refiltered through a 0.2 µm diameter filter membrane (Kutnjem et al., 2018b) and then stored in a bottle in the refrigerator at a temperature of 4°C. The extract was taken out of the refrigerator to perform the experiment half an hour before use.

For this study, two mixtures were made: (1) 500 g of compost was mixed with 1000 ml of sterilised water in a one-and-a-half-litre PE bottle with an open neck; (2) then 200 g was also mixed with 1000 mL of sterilised water in a one-and-a-half-litre PE bottle with an open neck. Each mixture was then shaken manually for two (02) minutes and incubated at room temperature ( $27^{\circ}$ C). After 3 and 7 days of incubation (extraction time), each mixture was filtered through a muslin cloth. Each extract obtained was refiltered through a 0.2 µm diameter filter membrane (Kutnjem *et al.*, 2018a).

A portion of each extract obtained underwent autoclave sterilisation for one hour at 121°C with the aim of eliminating any pathogens. Each autoclaved extract was coded 'sterilised extract', the other remaining part that was not autoclaved was coded 'non-sterilised extract'. These two types of extract were each stored in a bottle in the refrigerator at a temperature of 4°C, and then later subjected to tests to inhibit the mycelial growth of *H. turcicum*.

#### Experimental protocol for the in-vitro test

The test was conducted in Petri dishes, with concentrations of 2.5, 5 and 10% which consisted of taking 0.5, 1 and 2 mL of sterilised and nonsterilised compost extract mixed respectively in 19.5, 19 and 18 mL of still liquid PDA to give a final volume of 20 mL per Petri dish. After shaking, the mixture (PDA + compost extracts) was aseptically poured into 90 mm diameter Petri dishes in a laminar flow hood. Two controls were produced under the same conditions. The positive control (T+) received only 20 ml of PDA culture medium and the negative control (T-) received a mixture of 0.5 mL of fungicide (Ridomil Gold plus 66WP) and 19.5 mL of PDA culture medium (2.5% concentration).

After the medium had solidified, 7 mm diameter mycelial discs from young *H. turcicum* cultures were placed in the centre of the Petri dishes in the form of an agar pellet. Incubation took place at  $28^{\circ}$ C, under continuous light (Bouslim, 1996; Kutnjem, 2020). Each combination was repeated three times.

Measurement of the mycelial growth of the colonies began 48 hours after incubation, and 4 measurements of mycelial growth were taken on day 2, day 4, day 6 and day 8 until the petri dish of the positive control was invaded by *H. turcicum* mycelial filaments. Mycelial growth was then estimated by averaging two perpendicular diameters.

#### Evaluation of mycelial growth

The inhibition rate (I%) or percentage of inhibition due to each extract was assessed at day eight (8) after incubation in relation to mycelial growth in the control dishes according to the formula developed by Singh and Diwakar (1993). The inhibition rate (I%) or percentage of inhibition due to each extract was assessed at day eight (8) after incubation in relation to mycelial growth in the control dishes according to the formula developed by Singh and Diwakar (1993).

$$I(\%) = \frac{Dto(mm) - Dxi(mm)}{Dto(mm)} \times 100$$

I(%): percentage inhibition; Dto is the average diameter of the control batch and Dxi is the average diameter of the batches in the presence of the extract. The different diameters (D) were calculated according to the following formula (Singh and Diwakar, 1993):

$$D = \frac{D1 + D2}{2} - D0$$

Where: Do was the diameter of the explant; D1 and D2 were the diameters of the culture measured in the two perpendicular directions (Fig. 5).



Fig. 5. Diagram of mycelial growth measurement in Petri dishes on PDA medium.

The regression line showed whether or not there was a correlation between the percentage inhibition (PI) and the concentrations applied, depending on whether the correlation coefficient (r) of the line was close to unity (between 0.8 and 1) (Ngoh Dooh, 2014).

### Correlation between concentration and inhibition

The correlation between concentration and percentage inhibition was determined from the equation y = ax + b with x = concentration and y = percentage inhibition. The variable gave the slope; if a < 0, the slope is negative; if a > 0, the slope is positive. As for the correlation coefficient r, if it is between 0.8 and 1 then the correlation is perfect and positive; if r is between - 0.8 and -1 then the correlation is imperfect and negative (Heu *et al.*, 2012).

#### Determination of the Minimum Inhibition Concentration (MIC 50)

The minimum inhibition concentration of the aqueous extract of sterilised compost against *H. turcicum* was determined by comparing the value of the percentage inhibition with that of the natural logarithm of the corresponding concentrations (Ci):  $PI = f(\ln Ci)$ .

#### Statistical analysis

The data were first entered into Microsoft Excel (2017) after coding the results, and then the analysis of variance was carried out using SPSS 16.0 software. Means were compared at the 5% threshold using Duncan's test.

#### **Results and Discussion**

#### Influence of the compost extract production method on its ability to inhibit the mycelial growth of Helminthosporium turcicum

The various studies carried out around the world using compost extracts were difficult to compare, as the methods used to produce the extracts differ from one author to another. Two key parameters could then be involved in the production of the extracts: the water/compost extraction ratio and the extraction time. The two extraction ratios 1:2 and 1:5 (v:v; compost: water) tested in the present work did not influence the ability of the extracts to inhibit the mycelial growth of *H. turcicum*. The other important parameter in extract production was the extraction time. Varying the extraction time between 4 and 7 days did not influence the ability of the compost extracts to inhibit the mycelial growth of *H. turcicum*. For the two compost extracts used, the results obtained with these different ratios and extraction times did not differ significantly (P=0.983), with both extracts showing percentages of inhibition ranging from 74 to 96% (Fig. 6).



Fig. 6. Influence of the method of production of aqueous compost extract at a ratio of 1:2 (v:v; compost: water) (A) and a ratio of 1:5 (v:v; compost: water) (B) on their ability to inhibit the mycelial growth of *Helminthosporium turcicum* at a concentration of 2 ml.

These results facilitated the choice of a method for producing an aqueous extract of compost at a ratio of 1:2 (v:v; compost: water) after an extraction time of 4 days to continue the test for inhibiting the mycelial growth of *H. turcicum*.

#### Effect of sterilised aqueous extract (SAE) of household waste compost on mycelial growth of Helminthosporium turcicum

The mycelial growth of the *H. turcicum* strain under the control of the sterilised aqueous extract of household waste compost was greatly reduced.

Increasing the concentration considerably inhibited the growth of the strain to the order of 95.89% at concentration C3 (2 ml sterilised aqueous extract of compost). The results obtained as a function of the days after incubation showed significant differences (P=0.001) at the 5% threshold according to Duncan's test between the different concentrations and the controls tested. Fig. 7 shows the pictures of the mycelial growth of the strain at different doses on the eighth day after incubation and Fig. 8 shows а graphical representation of this growth.



Fig. 7. Growth of the *Helminthosporium turcicum* strain under the effect of sterilised compost extract on the 8th day after incubation.



Fig. 8. Changes in the diameters of *Helminthosporium turcicum* strain under the effect of sterilised compost extract.

(DAI = Day After Incubation; T + = Positive control containing only PDA culture medium; T - = Ridomil Gold plus 66WP; C1= medium concentrated with 0.5ml of aqueous compost extract; C2= medium concentrated with 1ml of aqueous compost extract; C3= medium concentrated with 2ml of aqueous compost extract. \*Values for the same day bearing different letters are significantly different at P < 0.05 (Ducan test)).

The statistical analysis showed that there was a significant interaction between the aqueous extracts (sterilised and non-sterilised) of compost and the *H. turcicum* tested. The results of the *invitro* test showed that the water/compost extraction ratio and the extraction time did not influence the ability of the aqueous compost extract to inhibit the mycelial growth of *H. turcicum*. Both extracts significantly reduced the radial growth of the *H. turcicum* strain, with a percentage inhibition of around 95.89% for the extract sterilised at concentration C3 (2 ml of aqueous compost extract) and 76.92% for the unsterilised extract.

Effect of unsterilised aqueous extract (USAE) of household waste compost on mycelial growth of Helminthosporium turcicum

The evolution of mycelial growth of the H. turcicum strain under the control of the unsterilised aqueous extract of household waste compost was greatly reduced. Increasing the concentration considerably inhibited the growth of the strain to the order of 76.92% at concentration C<sub>3</sub> (2 ml sterilised aqueous extract of compost). The results obtained as a function of the days after incubation showed significant differences (P=0.001) at the 5% threshold according to Duncan's test between the different concentrations and the controls tested. Fig. 9 showed an image of the mycelial growth of the strain at different doses on the eighth day after incubation and Fig. 10 showed a graphical representation of this growth.









(DAI = Day After Incubation; T + = Positive control containing only PDA culture medium; T - = Ridomil Gold plus 66WP; C1= medium concentrated with 0.5ml of aqueous compost extract; C2= medium concentrated with 1ml of aqueous compost extract; C3= medium concentrated with 2ml of aqueous compost extract. \*Values for the same day bearing different letters are significantly different at P < 0.05 (Ducan test))

Several suggestions emerge from these results: (1) the aqueous extract of household waste compost contains microorganisms that could be responsible for producing suppressive the substances responsible for the mechanisms inhibiting the mycelial growth of H. turcicum. Copeman et al. (2000) confirmed this assertion by isolating several antagonistic species of fungi and bacteria from extracts of household waste thev attributed which composts, to the suppressive effect of the extracts. However, similar results were obtained using a sterilised extract of household waste compost against Fusarium oxysporum. Other researchers have revealed that the mechanisms suggested for the activity of compost extracts on plant pathogens by are therefore induction of resistance stimulation of  $\beta$ -1.3-glucanase activity and direct inhibition of pathogens (Weltzeien, 1992; Zhang et al., 1998; Kavroulakis et al., 2005; De Corato, 2020; Curadelli et al., 2023). (2) The active

ingredient responsible for inhibiting *H. turcicum* mycelial growth could be heat-resistant compost substances or metabolites (Kutnjem, 2020; Bali *et al.*, 2021).

#### Comparative effect of sterilised and nonsterilised aqueous extracts of household waste compost on the mycelial growth of Helminthosporium turcicum

Analytical comparisons of the compost extracts tested showed that the sterilised extract had a slightly greater inhibitory effect than the nonsterilised extract. At the end of the experiment, the sterilised aqueous extract showed a percentage inhibition rate on the eighth day after incubation of around 95.89% at concentration C3. This inhibition rate was 18.97% higher than that of the non-sterilised aqueous extract at the same concentration and for the same duration, i.e. 76.92%. This comparison is shown graphically in Fig. 11.



Fig. 11. Illustration of the comparative effect of sterilised and non-sterilised aqueous extracts of household waste compost on the mycelial growth of *Helminthosporium turcicum*.

(DAI = Day after Incubation; SAE = Extract of sterilised compost; USAE: Extract of non-sterilised compost).

Larbi (2006) confirms this thermostable active principle in his Thesis. For Larbi, the active principles responsible for inhibiting pathogens, in particular Venturia inaequalis in apple seedlings and *Plasmopara viticola* in vine seedlings, are water-soluble and heat-resistant compost metabolites. Similar results were obtained by Cronin et al. (1996), Al-Dahmani et al. (1999), Abd-Alrahman and Aboud (2021). These authors point out that these water-soluble and heatresistant compost metabolites must already be relatively active at low concentrations, which explains why extraction ratios do not play a role in the effectiveness of the extracts. Cronin et al. (1996) were also able to demonstrate, in their mushroom compost extracts, that a low molecular heat-stable substance weight. was mainly responsible for inhibiting the germination of V. inaequalis conidia. However, according to these authors, this substance could be produced by anaerobic microorganisms during the incubation of the extract.

Correlation test between concentrations and inhibition percentages obtained with the different extracts The aim of this test was to see if there was a linear relationship between the decrease and increase in inhibition with the different concentrations of sterilised and non-sterilised aqueous extract of household waste compost on the radial growth of the *H. turcicum* strain. The regression lines obtained after analysis revealed similar behaviour of the strain with respect to the two extracts (sterilised and non-sterilised). All the lines obtained show negative slopes and perfect correlations between concentrations and the different percentages of inhibition, as shown in Fig. 12.

The regression equations obtained with the two extracts tested showed decreasing linear relationships with regression lines with negative slopes: y = -0.0005x+73.50 for the unsterilised extract; y = -0.00007x+84.36 for the sterilised extract. A perfect and negative correlation was obtained between the different concentrations and the percentage inhibition. The correlation coefficient was negative r = -1 for both extracts.



Fig. 12. Regression lines for mycelial growth in the different treatments (A= sterilised compost extract; B= non-sterilised compost extract).

#### Minimum inhibition concentration (MIC<sub>50</sub>)

From the regression lines obtained after the correlation tests, the concentrations of the two extracts inhibiting the growth of the *H. turcicum* strain by 50% were determined. The lowest inhibitory concentration (0.97 ml) was obtained with the sterilised aqueous extract of household waste compost. The highest  $MIC_{50}$  (3.45 ml) inhibitory concentration was obtained with the unsterilised aqueous extract of household waste compost.

In the present study, the substance responsible for inhibiting *H. turcicum* was already present in the compost, as the sterilisation of the compost extract did not damage its inhibiting power. However, the results do not allow us to say whether this substance comes from the decomposed organic matter or whether the microorganisms produce it during the composting process. Therefore, depending on the situation, different substances seem to be involved in the inhibition mechanisms. This is not surprising, given that the nature and characteristics of composts can greatly change. A more detailed or in-depth characterisation of these compounds could be a way of optimising the effectiveness of the extracts.

#### Conclusion

This research studied the in-vitro behaviour of compost extract on H. turcicum, a foliar disease that often leads to dry leaves and poor maize yields. The results show that compost extract, whether sterilised or not, has inhibitory properties in the laboratory that can halt the progress of the disease on the plant, opening the way to the production of biopesticides as supplements to plant protection products. To avoid jumping to conclusions, these interesting results hastv obtained in the laboratory need to be tested in experimental fields under real local conditions to better measure the effectiveness of compost extracts. Future work could be based on the hypothesis that the use of compost extracts to combat soil-borne diseases and to protect plant leaves against foliar pathogens are not techniques that can completely solve plant health problems, but they may have the potential to significantly reduce disease pressure. To build a good plant protection strategy, these techniques need to be combined with other production methods, in particular a combination of tillage methods, controlled fertilisation and irrigation, the use of specific antagonists, and a combination of compost in the soil and compost extract on the leaves. It is in this way that we will be able to put place a method of producing stable. in economically profitable and environmentally friendly plants for sustainable agriculture.

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