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Comparison between two methods of mycelia growth evaluation of some Oomycetes species

Najwa Benfradj*, Amani Rouini, Naima Boughalleb-M'Hamdi

Department of Biological Sciences and Plant Protection, High Institute of Agronomy of Chott Mariem, University of Sousse, 4042 Sousse, Tunisia

Abstract

Oomycetes pathogens causes devastating crop diseases worldwide. In this study, we compared mycelium growth of 7 Oomycetes species using the calculated area (CA) method obtained by perpendicular diameter of colony and measured area (MA) method. Results revealed a significant difference between Oomycetes species inhibition ratio using CA and MA, at lower mefenoxam concentrations. The interval of variation between MA/CA methods was variable according to Oomycetes specie. The largest interval (0.01-1000 µg/ml) was detected with *Pythium aphanidermatum* and *Phytopythium mercuriale*. The second interval (0.01-100 µg/ml) was determined by *Phytophthora cryptogea*, *P. aphanidermatum*, *P. ultimum* and *P. mercuriale*. An interval ranged from 0.01µg/ml to 31.6µg/ml was recorded with *P. cryptogea* and *Phytopythium vexans*. An interval from 0.01 µg/ml to 10µg/ml was determined with *Pythium dissotocum* and *P. vexans*; while the shortest interval (0.01-1µg/ml) was noted with *P. nicotianae*. In addition, present findings recorded a difference in Pearson's correlation index between growth factors (medium, evaluation dates, mefenoxam concentration) and Oomycetes mycelium growth using MA and CA methods. A statistical significance difference (P<0.05), weas noted more in case MA method and was more expressed in case of CA method.

Keywords: calculated area, measured area, growth factors, mefenoxam, ratio.

*Corresponding author: Najwa Benfradj,
E-mail: najwabenfradj@yahoo.fr

1. Introduction

Oomycetes are a group of microorganisms that shares microscopic hyphal morphology of fungi and contains large number of phytopathogens which cause disease in wide crop. Differences between Oomycetes and true fungi is that Oomycetes cell walls are composed of β-1,3 and β-1,6 glucans, whereas true fungi cell walls are composed of chitin (Williams-Woodward and De Mott, 2013). Fungi and Oomycetes mycelium grows out, on a surface of a natural substratum, by an expansion of spore or hypha results in a collection of a fungal colony. After an isotropic growth phase, hypha initiates random branching, forming fractal tree-like colonies (Fricker et *al.*, 2007). As for fungi, Oomycetes species growth is not easy to quantify because these organisms do not grow as single cells, but as hyphal filaments that cannot be quantified by the usual enumeration techniques (Taniwaki et al., 2006). Colonies shapes and surface textures provide useful information to determine species or to monitor growth state (Matsuura, 1999). Chemical control is the most used strategy to prevent Oomycetes diseases. Mefenoxam, is the active ingredient of a partially systemic fungicide largely used in the management of diseases caused by various Oomycetes (Olson et al., 2013). Mefenoxam inhibit ribosomal RNA synthesis of Oomycetes and thus suppressing the mycelial growth and the sporulation of the pathogen. *In vitro* evaluation of fungicide effect is the standard bioassay used to determine chemistries that influence growth of fungi and Oomycetes pathogens; in this assay, fungicide is amended into agar medium, and the response is recorded after a specified time interval (Noel et al., 2018). The fungal colony diameter is governed by the assumption that colony diameter is a good indicator of colony growth (Hendricks et al., 2017). Thus, measurement of radial growth is the most frequently method used as parameters to evaluate Oomycetes growth in this assay. Matsuura (1999) revealed that colonies shapes and surface textures provide useful information to determine the species or to monitor the growth state; colonies patterning looks to be highly sensitive to environmental factors, however, there might be underlying basic rules of pattern selection common for any species. However, Oomycetes species growth study are the heterogeneity of growth environments. In fact, morphological colonies of Oomycetes species could have different aspects such as uniform, stellate, radiate, petaloid/rosette and another forma according to medium and growth conditions. Furthermore, because of erratic developmental changes in the contents of such constituents such assays are generally not recommendable for assessing fungal growth inhibition (Broekaert *et al.*, 1990). In addition, due to the increasing availability of image-capturing techniques (Falconer *et al.*, 2010), an interesting alternative is the capturing images use because it's easy and does not require expensive equipment (De Ulzurrun *et al.*, 2015). The aim of this study was based on the fact that Oomycetes species have different colonies aspects, and that measurement of diameter colonies could not be the most suitable method to evaluate their growth state. Thus, the objectives of this investigation were to compare between calculated area (CA) obtained by perpendicular diameter and measured area (MA) recovered from image-capturing techniques to detect difference in colonies growth of some Oomycetes species.

2. Materials and methods

2.1 Isolates, culture conditions and inoculum preparation

Seven Oomycetes species isolates were investigated in this study. Two of these isolates belong to the genus *Phytophthora* (*P. nicotianae*, *P. cryptogea*), three belong to the genus *Pythium* (*P. ultimum*, *P. aphanidermatum*, *P. dissotocum*) and two were identified as *Phytopythium* spp. (*P.*

mercuriale, *P. vexans*). These isolates were recovered, between 2012 and 2013, from trunk and soil of citrus trees infected by gummosis and proved as the causative agents of this disease in Tunisia (Boughalleb-M'Hamdi et al., 2018; Benfradj et al., 2017). Initial cultures of isolates were prepared by transferring stored plugs of each one from conserved cultures in sterile soil solution onto PDA (Potato-Dextrose-Agar) medium plates. Before used, isolates were cultured for 6 days at 25±1°C, in darkness until mycelium covered the surface. The main characteristics of the used isolates are listed in Table (1).

Table 1: Characteristics of Oomycetes isolates obtained from infected citrus orchards by gummosis and used in the present study.

Species	Localities	GenBank Accessions numbers	Hosts
P. nicotianae	Takelsa	KU248808	*Thomson navel*
P. cryptogea	Hawaria	KU248814	*Clementine hernandina*
P. ultimum	Takelsa	KU248786	*Thomson navel*
P. aphanidermatum	Menzel bouzalfa	KU248783	*Clementine hernandina*
P. dissotocum	Bouargoub	KU248782	*Thomson navel*
P. mercuriale	Takilsa	KU248804	*Thomson navel*
P. vexans	Bnikhaled	KU248800	*Thomson navel*

2.2 Preparation of fungicide concentrations

The evaluation of response of Oomycetes species to mefenoxam (Ridomil Gold®EC, 96.2 % active ingredient, Novartis Crop Protection, Inc.) was assessed using the method of Pradhan et al. (2017). Thus, each fungicide concentration was prepared via serial dilutions in sterile distilled water in amber glass bottles, mixed on a stirring plate at medium speed for two minutes and used within 24 h. Minimum application rate (MAR) of mefenoxam (10.425 µg/ml) is used according to the estimation of Chen *et al.* (2001) who assuming soil depth and soil bulk density to be 2 cm and 1.2 g/cm^3, respectively. The first six concentrations of mefenoxam were from MAR × 10^3 to MAR × 10^{-3}, in 10-fold dilutions and the control (sterile distilled water) were used to determine the benchmark dose (BMD). BMD (≈100 µg/ml) was calculated using continuous Hill model, by default parameters via Benchmark Dose Software (BMDS, V. 2.7.0.4) (Flores and Garzon, 2013). BMD values were used to calculate the ten mefenoxam concentrations (BMD × 10^2, BMD × 10^{10}, BMD × $10^{0.5}$, BMD, BMD × $10^{-0.5}$, BMD × 10^{-1}, BMD × $10^{-1.5}$, BMD × 10^{-2}, BMD × $10^{-2.5}$, BMD × 10^{-3}) and prepared as a 10X stock solutions for the assays.

2.3 *In vitro* effect of mefenoxam doses on Oomycetes mycelial growth

Mycelial growth of Oomycetes isolates was evaluated onto PDA and CMA (Corn-Meal-Agar) (Tulip Diagnostics, Goa, India) medium. Used medium was prepared according to the manufacturer's instructions, dispensed in number of flasks (250 ml) equal to the number of treatments in each assay, and autoclaved at 120°C, for 20min. The medium was cooled down to 60°C, before adding each solution of fungicide concentration. Each medium-fungicide combination was stirred for two minutes using magnetic stirrers and poured into 9 cm-diam petri dishes containing 20 ml of each medium. After solidification, agar plugs of each isolate (5 mm in diameter) were placed on petri dishes center of medium amended with each fungicide concentration. Ten fungicide concentrations (1000, 100, 31.6, 10, 3.16, 1, 0.31, 0.1, 0.03 and 0.01 µg/ml) and a control without fungicide (0 µg/ml) were

used for each experiment. Petri dishes were sealed with parafilm and incubated at 25±1°C in the dark, for 6 days.

2.4 Methods of growth measurement

Every 2, 4 and 6 days of incubation, isolates growth was measured by calculated the colony diameters area (CA) and measured the surface area (MA) on PDA/CMA medium with or without fungicide concentration. Diameter area of each isolate was calculated from the reverse side of petri dish (90mm) in millimeters using a ruler. Mean diameter growth for each isolate was measured by averaging colony diameter measurements and subtracting the plug diameter. For MA, isolates images colony were scanned using CanoScan 8400 F (Canon, Melville, NY) and the whole area of the colony was measured using the software Statgraphics Plus 5.1.

2.5 Data analysis

For all experiments, three replications per isolate–fungicide concentration combination were carried, and each experiment was repeated twice in the time. Methods of measurement of Hendricks *et al.* (2017) were used to determine if there is a difference in analyzed results between CA and MA measurements and whether these differences changed the final conclusion made at the treatment level. Thus, the average diameter of each colony was converted to area of a circle using the following formula:

$$D_A = \pi r^2 = \pi(\frac{\text{Average D}}{2})^2$$

With: D_A: Diameter converted to area of circle; D: average diameter of each colony.

Then, the ratio of mycelia growth inhibition of each isolate by the fungicide were calculated according to the formula:

$$\text{Ratio} = \frac{(\text{Average Control Area} - \text{Average Treatment Area})}{\text{Average Control Area}}$$

For the comparison between the two methods of evaluation, obtained data were subjected to variance analysis (ANOVA) and multiple comparisons of means was carried using Student-Newman-Keuls test ($P<0.05$) as a post hoc test (XLSTAT 2018.1. Software). In addition, Principal component analysis (PCA) at $P < 0.05$, with a Pearson correlation coefficient at $n = 1$ were used to identify the variation in Oomycetes species inhibition ratio, in each evaluation method, across the growth factors (STATISTICA 13.5.0.17).

3. Results

3.1 Evaluation of two methods of *in-vitro* growth of Oomycetes species

Results of this study proved the growth suppressive effect of mefenoxam on Oomycetes species and the existence of difference between two methods of growth evaluation of these species. Both, using PDA and CMA medium. The suppressive effect of mefenoxam increase according to the evaluation dates, in all Oomycetes species. No difference between MA/CA methods was noted at higher mefenoxam concentrations, because of the complete inhibition of Oomycetes mycelium growth (Ratio= 1). However, MA method was more efficient than CA method to determine subtle changes in Oomycetes mycelia growth at variables intervals of lower mefenoxam concentrations. Intervals of variation between MA/CA methods were influenced by Oomycetes species growth factors (medium, dates, concentrations).

3.1.1 Case of *Phytophthora* species

Results recorded unequal interval of variation between CA/MA methods using *Phytophthora* species. For *P. nicotianae,* this interval was between the concentrations 0.01 µg/ml and 1 µg/ml for both used medium. At this interval, inhibition ratio was higher using PDA medium (Figure 1a) then by CMA medium (Figure 1b) (ANOVA, for Ratio $_{MA}$ (F)= 30.571/ for Ratio $_{CA}$ (F)= 8.435, $p < 0.004$). Inhibition ratio of *P. nicotianae* was ranged from 0.268 to 0.973 using MA method compared to an interval from 0.128 to 0.829 by CA method. However, MA/CA interval of variation of *P. cryptogea* was largest then *P. nicotianae* one but associated to used medium. In fact, using PDA medium, this interval was ranged between the concentrations 0.01 µg/ml and 31.6 µg/ml (Figure 1a), while using CMA medium, it was between 0.01 µg/ml and 100 µg/ml (Figure 1 b). At this interval, no difference was noted between inhibition ratio of *P. cryptogea* in PDA and CMA medium.

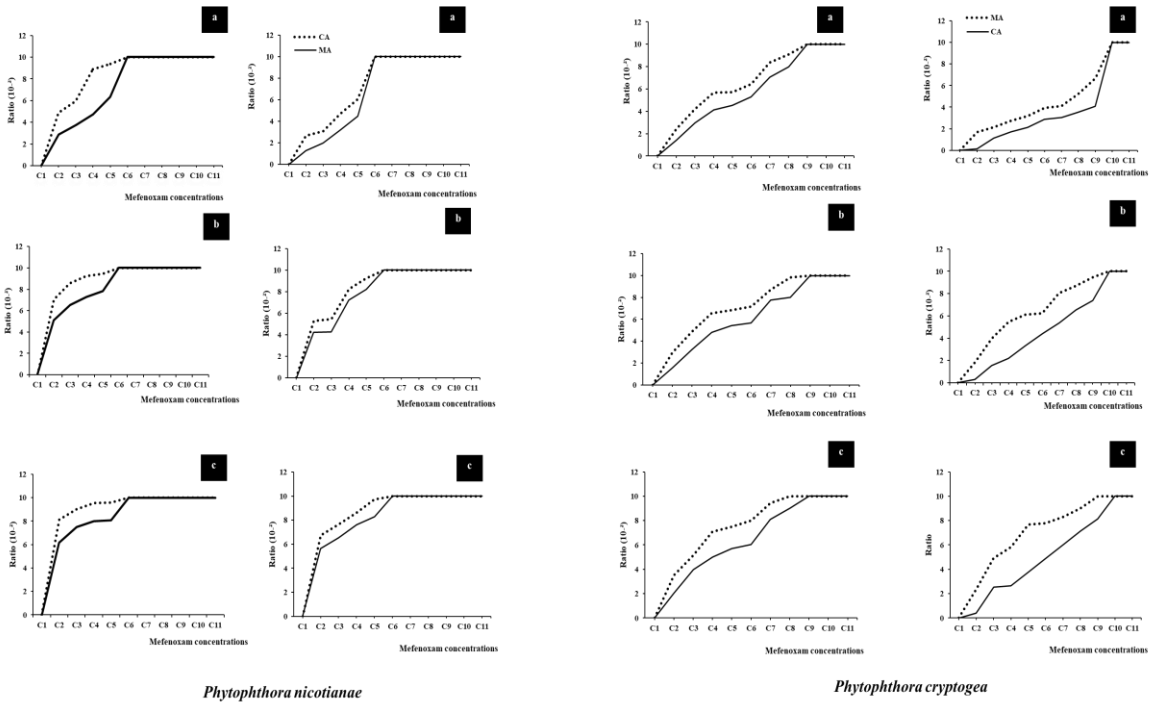

Figure 1: Comparison between calculated area (CA) and measured area (MA) of *Phytophthora nicotianae* and *P. cryptogea* using PDA and CMA medium, amended with different concentrations of mefenoxam after 48 (a), 96 (b) and 144h (c) of incubation at 25±1°C, in darkness.

Inhibition ratio of *P. cryptogea* was from 0.168 to 1 using MA method compared to an interval from 0.011 to 0.901 by CA method. At these intervals, 'Medium-Concentration-Day' effect was highly significant between Ratio $_{MA}$ and Ratio $_{CA}$, having p-values of $p<0.0001$ in case of *P. nicotianae* (for Ratio $_{MA}$, F= 5.591/ for Ratio $_{CA,}$ F= 5.270) and *P. cryptogea* (for Ratio $_{MA}$, F= 10.040/ for Ratio $_{CA,}$ F= 5.903).

3.1.2 Case of *Pythium* species

The largest interval of variation between MA/CA methods was obtained with *Pythium aphanidermatum* grown onto CMA medium (0.01 µg/ml – 1000 µg/ml). The second

interval of variation (0.01 µg/ml – 100 µg/ml) was noted with *Pythium ultimum* using both medium and in case of *Pythium aphanidermatum* using PDA medium. However, with *Pythium dissotocum*, the interval of variation between MA/CA methods was between 0.01 µg/ml and 10 µg/ml. Inhibition ratio of *P. ultimum* was from 0.123 to 1 using MA method compared to an interval from 0.083 to 0.857 by CA method. Inhibition ratio of *P. aphanidermatum* was from 0. 213 to 1 using MA method compared to an interval from 0.100 to 0.812 by CA method. However, inhibition ratio of *P. dissotocum* was from 0. 179 to 0.980 using MA method compared to an interval from 0.050 to 0.910 by CA method (Figure 2). A high significant difference was noted in ration between PDA and CMA medium, using *Pythium aphanidermatum* (ANOVA, Ratio $_{MA}$ (F)= 11.903/ for Ratio $_{CA}$ (F)= 11.478, $p<0.001$). However, no differences among medium used were recorded neither for *Pythium ultimum* nor *Pythium dissotocum*. Furthermore, 'Medium-Concentrations-Day' effect was highly significant between Ratio $_{MA}$ and Ratio $_{CA}$, having p-values of $p<0.0001$ in case of *P. ultimum* (for Ratio $_{MA}$, F= 7.235/ for Ratio $_{CA,}$ F= 19.470), *P. aphanidermatum* (for Ratio $_{MA}$, F= 11.715/ for Ratio $_{CA,}$ F= 6.121) and *P. dissotocum* (for Ratio $_{MA}$, F= 10.463/ for Ratio $_{CA,}$ F= 5.267).

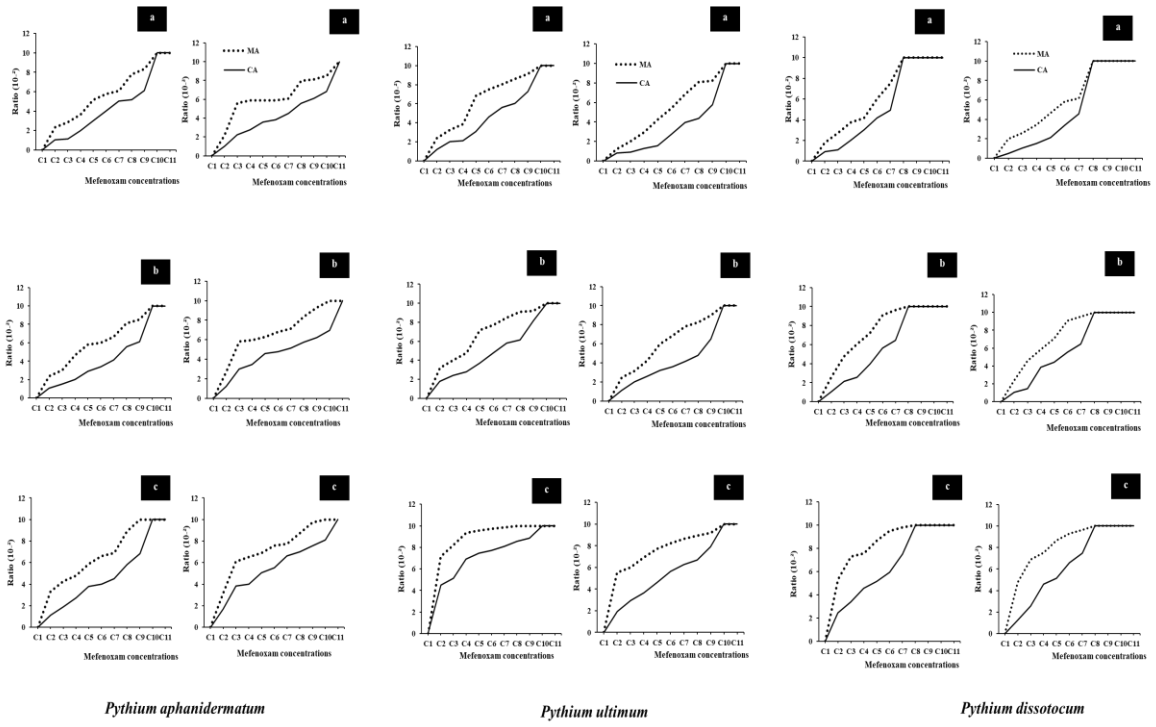

Figure 2: Comparison between calculated area (CA) and measured area (MA) of *Pythium aphanidermatum*, *P. ultimum* and *P. dissotocum* using PDA and CMA medium, amended with different concentrations of mefenoxam after 48 (a), 96 (b) and 144h (c) of incubation at 25±1°C, in darkness.

3.1.3 Case of *Phytopythium* species

For *Phytopythium* species, the interval of the variation between MA and CA methods was also variable according to the species and depending on used medium. For *Phytopythium mercuriale*, this interval was from 0.01 µg/ml to 100 µg/ml using PDA medium and from

0.01 µg/ml to 100µg/ml using CMA medium. However, for *Phytopythium vexans*, the interval was from 0.01µg/ml to 10µg/ml using PDA medium and from 0.01 µg/ml to 31.6 µg/ml using CMA medium (Figure 3). A significant difference of inhibition ratio noted between PDA and CMA medium, using *Phytopythium mercuriale* (ANOVA, Ratio $_{MA}$ (F)= 22.695/ for Ratio $_{CA}$ (F)= 7.94146, p<0.005).

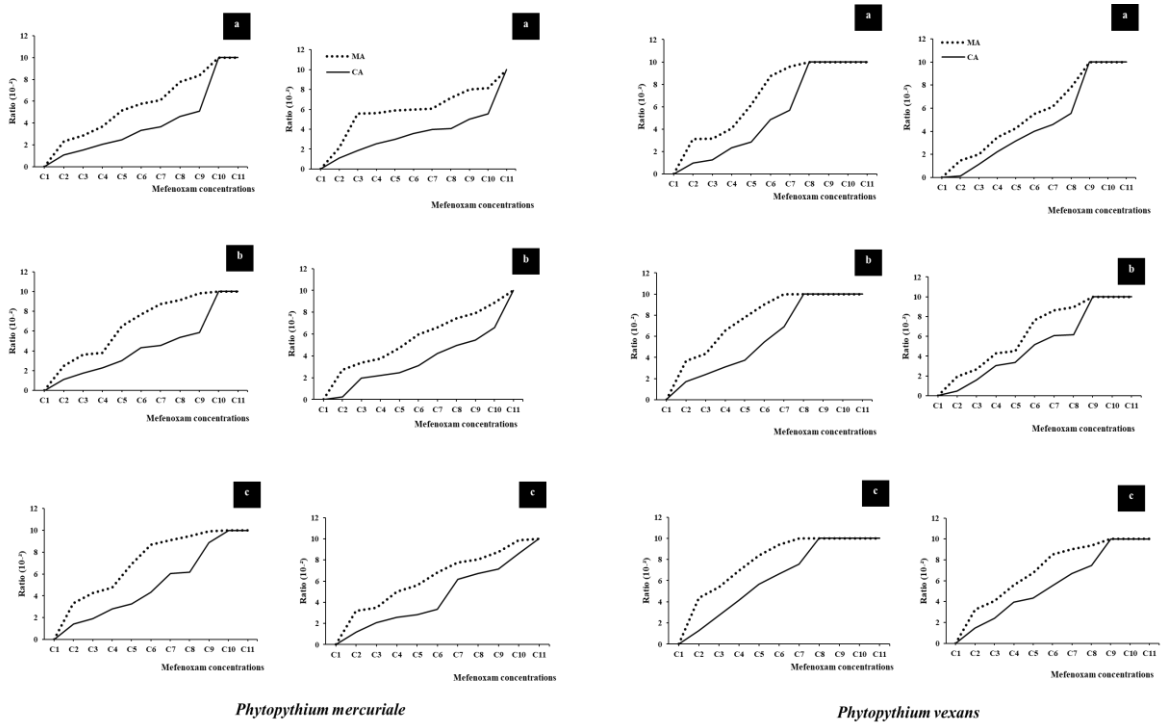

Figure 3: Comparison between calculated area (CA) and measured area (MA) of *Phytopythium mercuriale* and *P. vexans* using PDA and CMA medium, amended with different concentrations of mefenoxam after 48 (a), 96 (b) and 144h (c) of incubation at 25±1°C, in darkness.

Phytopythium vexans revealed a significant difference between the two-medium used just in case of CA method (ANOVA, Ratio $_{MA}$ (F)= 5.362/ for Ratio $_{CA}$ (F)= 7.941, p<0.02). Inhibition ratio of *P. vexans* was from 0. 146 to 1 using MA method compared to an interval from 0.010 to 0.100 by CA method, while inhibition ratio of *P. mercuriale* was from 0. 213 to 0.992 using MA method compared to an interval from 0.106 to 0.887 by CA method. Also, the effect 'Medium-Concentrations-Day' was highly significant between Ratio $_{MA}$ and Ratio $_{CA}$, having p-values of p<0.0001 in case of *Phytopythium mercuriale* (for Ratio $_{MA}$, F= 6.251/ for Ratio $_{CA,}$ F= 6.803), and *Phytopythium vexans* (for Ratio $_{MA}$, F= 8.773/ for Ratio $_{CA,}$ F= 22.426).

3.2 Correlation between growth factors and inhibition ratio of Oomycetes species

Regardless of the clear site separation detected by the Principal Component Analysis in both evaluation methods, a markedly differences were noted. In MA method, the first component (PCA1) explained 72.540 of the sites variances and was mainly determined by medium used. The second component (PCA2) captured 10.575 of variance of site distribution and was determined by the dates and the

mefenoxam concentrations. The same components determination was obtained for CA method. However, in this method, PCA1 explained 72.747% of sites variance, and PCA2 captured 10.666%. Both using MA and CA methods, growth factors and pathogens inhibition ratio were grouped in the positive end of PCA1. However, changes were recorded in PCA2. In fact, for MA method *Phytophthora* species, *P. ultimum* and *P. vexans* were located in the positive end of PCA2. However, *P. aphanidermatum, P. dissotocum* and *P. mercuriale* were located on the negative end of PCA2. In the other hand, by CA method, all *Phytophthora* and *Phytopythium* species, in addition with two *Pythium* species (*P. ultimum*, *P. dissotocum*) were located on the positive end of PCA1. Grouped on the opposite PCA1 end was just the pathogen *P. aphanidermatum* (Figure 4). In comparing the matrix cubensis expression profiles of MA and CA methods, a difference in Pearson's correlation index (*r*) among inhibition ratio of Oomycetes species and growth factors was noted. In MA method, using medium the majority of Pearson's correlation index values were between -0.273 and -0.091, while values this correlation was higher in case of CA method, ranged between -0.091 and 0.091. However, using mefenoxam difference between the degree of correlation was noted just in case of *P. nicotianae* where it was high in case of CA method (0.818-1) than in case of MA method (0.273-0.455). In addition, according to evaluation dates, difference was noted in case of *P. vexans* with higher correlation using CA method (0.091-0.273) than using MA method (-0.091-0.051).

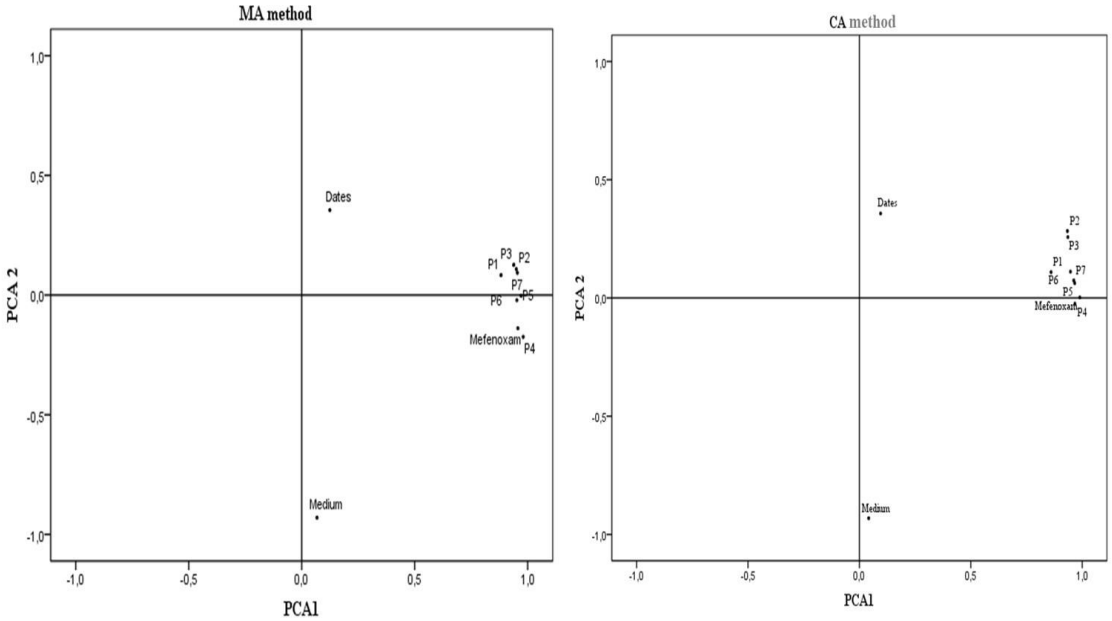

Figure 4: Difference among Principal components analysis (PCA) scatterplot between the grown factors and inhibition ratio of Oomycetes species using MA and CA methods (P1: *P. nicotianae*; P2: *P. cryptogea*; P3: *P. ultimum*; P4: *P. aphanidermatum*; P5: *P. dissotocum*; P6: *P. mercuriale*; P7: *P. vexans*). Black dots indicate average measures of each factor.

A scatter plot matrix was generated by performing the correlation matrix analysis based on linear regression depicts the influence of growth factors on Oomycetes growth in each method of evaluation. Values with statistical significance at the level P > 0.05

were noted more in case of MA method then in case of CA method. In fact, with AM method, *P. nicotianae* (-0.107) and *P. aphanidermatum* (0.087) were found to be correlated to medium, while *P. mercuriale* (0.109) shown to be positively correlate with dates. However, by CA method, just a one positive correlation was found between dates and *P. aphanidermatum* (0.100). In addition, Pearson's correlation index (*r*) has values with statistical significative at the level P >0.01 for both methods. In MA method, it is evident that medium showed a good positive linear relation (r = 0.87) with *P. aphanidermatum*, a negative linear relation with *P. nicotianae* (-0.107), *P. cryptogea* (-0.156), *P. ultimum* (-0.133), *P. vexans* (-0.157). However, the correlation values obtained for *P. dissotocum* (-0.015) and *P. mercuriale* (-0.076) were insignificant. In the other hand, using CA method just a negative linear correlation was made between medium and *P. cryptogea* (-0.238) and *P. ultimum* (-0.164), while insignificant values were noted with other species. In addition, mefenoxam exhibited a notable positive linear correlation with all Oomycetes species using both methods. Interestingly, except *P. nicotianae* (r=0.743), all Oomycetes species shown a positive linear correlation (r>0.818). Further, as shown, evaluation dates, exhibited a moderately linear positive correlation with all Oomycetes species (r>0.01) in case of MA method. However, a in case of CA method, a moderately linear positive correlation with all Oomycetes species (r>0.01) were noted from *P. nicotianae*, *P. cryptogea*, *P. ultimum*, *P. dissotocum*, *P mercuriale*; while a low linear positive correlation was noted with *P. vexans* (r=0.047).

4. Discussion

The present work compares two methods of evaluation of Oomycetes species mycelium growth. Results of this study showed that method of measured area was more efficient then calculated area method to distinguish differences in fungicide growth inhibition for Oomycetes species. In fact, measured area was more efficiency to distinguish subtle changes in Oomycetes growth inhibition at low concentrations of mefenoxam. Our results agree with Hendricks et al. (2017) results who revealed that calculated area and/or measured area was adequate to distinguish significant treatment effects of fungicide on fungal growth, however MA was more sensitive. In fact, in study of these authors noted that analysis of area calculated from colony diameter and measured area were adequate to distinguish significant differences in fungicide growth inhibition for *Phyllosticta citricarpa* at lower fungicide concentrations. Difference between the methods of evaluation found in this study, could be due to that Oomycetes hyphae are not grow completely straight and produce extent. In fact, fungal growth and direction occurs as the combined result of various biological processes, such as the ubiquity and ecological impact (Riquelme et al., 1998), environmental stimuli (Graham, 1995). Much research was done on the ability of hyphae to change growth direction (tropisms) in response to external stimuli (Riquelme et al., 1998). Measurement of the growth rate-nutrient level relation for the fungal strains is expected useful to understand the diversity in the colony patterning under the change of other environmental factors, such as the stiffness of the substrate (Matsuura, 1999). According to Riquelme et al. (1998), hyphae elongate by apical growth, polarized and growing tip is the likely place where the growth directionality of a hypha is established. In fact, the fungi ability to generate polarized cells with a variety of shapes reflects a temporal and spatial control over the polarity axes formation (Riquelme et al., 2011). Angle

at which a new hypha branches relative to the existing one depends on the species (Kamel et al., 2009). Also, by growing and exploring, a hypha can encounter another hypha and fuse with it (anastomosis), as such changing the fungal network shape and increasing the nutrient cycle efficiency (Simonin et al., 2012). Hyphal apex shape and diameter are results of the relative rates of wall component synthesis and rigidification (Graham, 1995). Oomycete cell wall is composed of β-1,3, and β-1,6 glucans, and not of chitin (William et al., 2010). Growing hyphae of fungi have appreciable activities of chitinases and other lytic enzymes (Graham, 1995). The spitzenkörper position in the hyphal tip determines the growth direction (De Ulzurruna et al., 2017; Lopez- Franco and Bracker, 1996; Girbardt, 1957). CA method limitations could be explained by the fact that chitin content of hyphae may change with age and growing conditions (Matcham et al., 1985). Gooday (1976) noted that difference in Oomycetes colonies behavior may be explained by the fact that their hyphae respond chemotropically to nutrients such as sugars. In our study, the composition and the nutrient in the two media used are different. In fact, for Oomycetes species, CMA media have more nutriment than PDA media. According to Matsuura (1999), hyphae were produced densely inside the colony and oppositely at high nutrient level, while at low nutrient levels colonies expand to cover almost entire medium surface with far less hyphal density. Measurement of fungal strains growth rate-nutrient level relation is expected useful to understand the diversity in the colony patterning under the change of other environmental factors, such as the stiffness of the substrate (Olsson, 2001). According to Trinci (1969), when comparisons are made between species colony radial growth rate is not a meaningful parameter of growth. In fact, in his study, Trinci (1969) found that if the environmental conditions within this peripheral growth zone became unfavorable to growth, the colony radial growth rate would decrease; thus, this author affirm that colony radial growth rate is not a reliable parameter of specific growth rate in submerged culture in studies where nutrient concentration is varied. Results of our study showed differences between colony morphology of Oomycetes used. Also, Taniwaki et al. (2006) and Schnürer (1993) noted differences in growth patterns between different fungal species. Olsson (2001) colony morphology and medium could change due to fungal activities. Mechanism behind this measurments efficient is probably the ability of MA to distinguish small changes in fungal growth inhibition. In addition, one of the main advantages of the image analysis method presented is that it is completely automated does not require direct interaction with the samples, allowing to follow the entire fungi growth (De Ulzurrun et al., 2015). The image gamma correction applied is important to enhance the contrast between the object of interest and the background, because the colony area of the is completely isolated from the remaining image (Da Silva et al., 2017). By using proper masks on the original images, it could be possible to study local behavior within the colony, such as the difference in growth behavior and morphology between the central and the peripheral region where hyphae avoid contact and generate less branches and fusions (Riquelme et al., 2011). However, this method of measurement needs a high contrast picture of the colonies. In the other hand, although CA method shown to be an easy and good measurement approach, only horizontal growth is considered in this method. CA method doesn’t take in consideration the pathogen vertical growth or the density increase in the Petri dish. Also, a drawback of using two-dimensional measures such as area

or diameter is that the third colony density dimension is not taken into consideration (Taniwaki et al., 2006). In addition, interactions between fungi and their environment are often neglected (De Ulzurruna et al., 2017). A difference was also noted between MA/CA methods correlation matrices. Overall, using both methods a negative correlation was mentioned between pathogens and medium used. However, a positive correlation was noted between pathogens and mefenoxam and evaluation dates. Results classified the degree correlation of factors as high in CA method compared to MA one. This could be explaining by the low growth rate obtained in case this method compared to MA one. According to Miyashira et al. (2010) and Loeck *et al.* (2004), the low growth rate has been considered as a limiting factor regarding several experimental analyses.

5. Conclusion

The present work compares two methods of evaluation of Oomycetes species mycelium growth. Results of this study showed that method of measured area was more efficient then calculated area method to distinguish differences in fungicide growth inhibition for Oomycetes species. In fact, measured area was more efficiency to distinguish subtle changes in Oomycetes growth inhibition at low concentrations of mefenoxam.

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