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*Research Paper*

# *In vitro* efficacy of fungicides for control of *phytophthora nicotianae* a causal organism of pineapple heart rot disease in Uganda

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Pineapple heart rot disease (PHRD) caused by *Phytophthora nicotianae* is a threat to pineapple industry in Uganda. Currently, there is uncertainty in the management of the disease. Elsewhere, fungicides are used for management of *Phytophthora* diseases. The objective of this study was to determine *in vitro* efficacy of fungicides for the control of *Phytophthora nicotianae* causing PHRD. Twenty isolates of *Phytophthora nicotianae* were tested for sensitivity to Metalaxyl, Victory 72 powder and Fosetyl Al on 10% V8 media at 0.1g $L^{-1}$, 0.01 $L^{-1}$ and 0.001 g $L^{-1}$ *in vitro*. All isolates were sensitive to Metalaxyl and Victory 72 powder at 0.1 g $L^{-1}$ with no growth in the plates. However, there was a significant ($P<0.05$) difference in isolate growth at 0.01 and 0.001 g $L^{-1}$ Metalaxyl and Fosetyl Al. At 0.01 g $L^{-1}$ Fosetyl Al, the highest and lowest colony diameters were 71.89 mm (MAS 01) and 17.61 mm (MUK 01) compared to 77.44mm (MAS 01) and 0.00mm (MUK 01; MAS 02) in Metalaxyl amended plates respectively. Based on this result, it was concluded that Metalaxyl and Victory 72 powder were effective in suppressing *Phytophthora nicotianae*. However, determining the efficacy of these formulations under field conditions is recommended.

**Key words:** Pineapple, Heart rot, *Phytophthora nicotianae, In vitro,* Efficacy, Fungicides

## INTRODUCTION

*Phytophthora nicotianae* is a destructive pathogen causing pineapple heart rot disease in several parts of the world (Shen *et al.,* 2013; Rodríguez *et al*., 2015; Shreenivasa *et al*., 2015; Ocwa *et al*., 2016). According to Rodriguez *et a*l. (2015), pineapple heart rot disease is widespread and devastating leading to huge economic. In Uganda, pineapple heart rot disease is reported and confirmed in the central districts of Masaka, Mukono, Luwero and Kayunga (Ocwa *et al*., 2016). However, the information on effective management of the causal organisms is limited and scanty (NARO, 2012). Yet, elsewhere, chemicals are effectively used to control Oomycetes like *Phytophthora* spp (Hu *et al.,* 2008; Nina, 2014). For example, Metalaxyl and Fosetyl Al are some ofthemajorchemicalsusedformanagemenof *Phytophthora* diseases in plants (Jeffers and Miller, 2001). However, due to the repeated use, Metalaxyl is prone to develop resistance (Jeffers and Miller, 2001). Therefore, proper concentrations have to be used to prevent resistance and environmental effect. According to Mihajlovic *et al*. (2011), Fosetyl Al and Propamocarb hydrochloride have good efficacy against some diseases caused by several *Phytophthora* species. However, Fosetyl Al has a low activity against mycelia growth *in vitro* (Fenn and Coffey, 1983; Rorhbach and Schenke, 1985). On the other hand, in India, a study by Padmaja *et al*. (2015) revealed that Metalaxyl (2.5g $L^{-1}$) reduced *in vitro* growth of *Phytophthora nicotianae* by 92.9%. Therefore, proper understanding of pathogen sensitivity to chemical compounds has effect on disease management since

**Table 1**. Origin of isolates used in the study.

| Isolate | District* |
|---|---|
| KAY 01 | Kayunga |
| KAY 02 | Kayunga |
| KAY 03 | Kayunga |
| KAY 10 | Kayunga |
| KAY 13 | Kayunga |
| KAY 14 | Kayunga |
| LUW 03 | Luwero |
| LUW 05 | Luwero |
| LUW 09 | Luwero |
| LUW 12 | Luwero |
| LUW 15 | Luwero |
| LUW 08 | Luwero |
| LUW 06 | Luwero |
| LUW 14 | Luwero |
| MUK 01 | Mukono |
| MUK 02 | Mukono |
| MUK 03 | Mukono |
| MAS 01 | Masaka |
| MAS 02 | Masaka |
| MAS 03 | Masaka |

* KAY-Kayunga, LUW-Luwero, MUK-Mukono, MAS- Masaka

insensitivity may result into failure in disease control (Kimberly *et al.,* 2010).

In Uganda, a variety of fungicides are reportedly used for managing most fungal diseases (Tusiime, 2014). For example, Victory 72 powder which is a new fungicide in the market with similar active ingredient with Metalaxyl is widely used.

However, little is known about the sensitivity of *P. nicotianae* to Metalaxyl, Fosetyl Al and Victory 72 powder in Uganda. Hence information on fungicide reaction is critical to developing management strategies (Hu *et al*., 2008; Akrofi, 2015). The aim of this study, therefore, was to determine *in vitro* efficacy of fungicides for the control of *Phytophthora nicotianae* causing pineapple heart rot disease in Uganda.

## MATERIALS AND METHODS

### Experimental location

The study was conducted at National Agricultural Research Laboratories, Kawanda (NARL) along Bombo road, 13 km north of Kampala between April to August 2016

### Isolates, Fungicides, and Fungicide concentrations used in the study

Twenty *Phytophthora nicotianae* isolates used in the study were recovered from samples of symptomatic pineapple leaves collected from districts of Kayunga, Luwero, Mukono and Masaka (Table 1). Isolation and pathogen confirmation was done as described by Drenth and Sendall (2001).Three fungicides namely, Metalaxyl [Methyl N-(methoxyacetyl)-N-(2, 6-xylyl)-DL-alaninate], Victory 72 powder (Metalaxyl 8% + Mancozeb 64%), and Fosetyl Al [(Aluminum tris (O-ethyl phosphonate)] were used during the study. Initially, Metalaxyl and Victory 72 powder were compared at 0.1g $L^{-1}$ but all the 20 isolates were suppressed. Because of this, eight representative isolates were tested on Metalaxyl and Fosetyl Al (Substituted Victory 72 powder) fungicides at a concentrations of 0.01 and 0.001g $L^{-1}$, respectively. The experiment was repeated twice.

### Media preparation and amendment

V8 media (10%) was prepared as described by Jeffers (2006). The cooled autoclaved media was then amended with appropriate quantities of the fungicides (Hu *et al*., 2008). Each of the 20 *Phytophthora* isolates was grown on 10% V8 agar in petri plates for 7 days at 25°C (Kimberly *et al.,* 2010).

Thereafter, a 5 mm diameter mycelial disc was taken from fungal culture and transferred to the centre of a petri plate containing 10 ml of clarified V8 agar amended with fungicides Metalaxyl and Victory 72 powder at a concentration of 0.1g $L^{-1}$ (Metalaxyl and Victory 72 powder) and 0.01 and 0.001 g $L^{-1}$ (Metalaxyl and Fosetyl Al). Control plates without fungicides were included in the study.

Culture plates for each isolate was arranged in complete randomized design (CRD) and replicated three times.

### Data collection

Colony measurements were recorded on the third, fifth and sixth day after transfer. This was done by measuring the diameter of the colony and subtracting 5mm to correct for the plug length. Measurement did not go beyond the $6^{th}$ day because some isolates had already covered the petri plate **(**Al-Masri *et al*., 2015). Isolates were scored as resistant or sensitive (Hu *et al*., 2008) depending on whether or not they grew in 0.1 g $L^{-1}$ Metalaxyl. Percentage inhibition for each isolate was calculated as the difference between the mean colony diameter of fungicide amended plates and control plates divided by the colony diameter of control plates expressed as a percentage (% ratio) (Bekker *et al*., 2005), mathematically expressed as:

Percentage inhibition= (C-T) x 100/ C

Where:

C = colony diameter (mm) on the control plate.
T = colony diameter (mm) on the test plate.

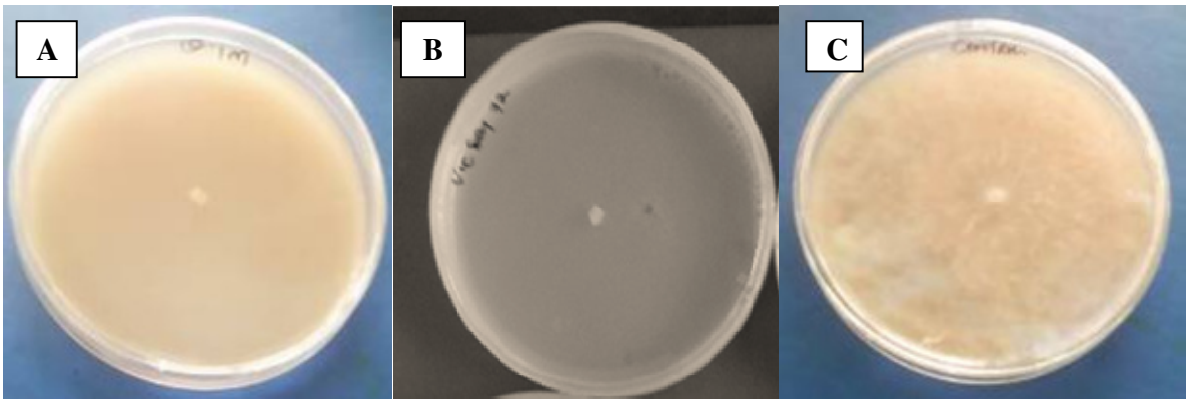


Figure 1. Complete suppression of mycelial growth of *Phytophthora nicotianae* isolates by Metalaxyl and Victory 72 powder) A) Complete suppression at 0.1g $L^{-1}$ Metalaxyl on the seventh day of incubation. B) Complete suppression at 0.1g $L^{-1}$ Victory 72 powder on the seventh day of incubation. C) Control plates with *Phytophthora nicotianae* isolate growth.

### Data analysis

Data on colony diameters was analyzed using analysis of variance of the Genstat computer programme (15th edition) (Bekker *et al*., 2005). Significant differences between means were separated using the Least Significant Different (LSD) at 5% probability level.

## RESULTS AND DISCUSSION

There was no significant difference (P>0.05) in growth of isolates from the media amended with Metalaxyl and Victory 72 powder at 0.1 g $L^{-1}$, respectively. In fact, all the isolates from all the districts were sensitive to Metalaxyl and Victory 72 powder at 0.1 g $L^{-1}$. In fact, no fungal growth was observed on V8 media amended with Metalaxyl and Victory 72 powder as opposed to the control during the trial period (Figure 1). However, there was significant difference (P<0.05) in the growth of isolates when concentrations of the amended media were reduced to 0.01 and 0.001 g $L^{-1}$, respectively. The highest and lowest colony diameters both concentrations were recorded from Fosetyl Al and Metalaxyl amended media, respectively (Figure 2), were 71.67 mm (MAS 01) and 13.67 mm (LUW 14) compared to 71.33mm (MAS 01) and 7.50 mm (LUW 14) in Metalaxyl amended plates respectively. Accordingly, at 0.01g $L^{-1}$ Fosetyl Al, the highest and lowest colony diameters were 71.89 mm (MAS 01) and 17.61 mm (MUK 01) compared to 77.44mm (MAS 01) and 0.00 mm (MUK 01; MAS 02) in Metalaxyl amended plates. In general, isolates KAY 01, KAY 14, MUK 01, MUK 02 and MAS 02 had the lowest colony diameters with complete suppression at 0.01g $L^{-1}$ Metalaxyl concentration opposed to isolate MAS 01 (Figure 2). In terms of inhibition, Metalaxyl at concentration 0.001 and 0.01g $L^{-1}$produced highest mycelial growth inhibition of isolates as opposed to Fosetyl Al (Figure 3). Overall, low mycelial growth was observed in all Metalaxyl amended plates irrespective concentrations compared to plates amended with Fosetyl Al (Figure 4).

Complete suppression of mycelial growth at 0.1 g $L^{-1}$ is an indication that Metalaxyl and Victory 72 powder can be used in management of pineapple heart rot disease. In fact, Victory 72 powder a new fungicide in the market can be used for controlling pineapple heart rot disease in absence of Metalaxyl which is normally scarce. According to Fontema *et al.* (2005), sensitivity of *Phytophthora nicotianae* isolates is attributed to low selection pressure since the pathogen was not exposed to excessive use of chemicals. However, the reason for less sensitivity of isolate MAS 01 to low fungicides concentrations was not clear but could be due to a mutation in the pathogenicity gene of this isolate. This aspect needs further investigation to be conclusive. Some studies have pointed the geographical influence on sensitivity of isolates to certain fungicides. Fontema *et al.* (2005) reported that in Cameroon Metalaxyl sensitivity was significantly influenced by geographic origin of the isolates because in certain regions, farmer used excessive chemicals resulting into resistance.

From the results, Fosetyl Al did not suppress *Phytophthora nicotianae* in comparison to the other fungicides did. According to Fenn and Coffey (1983), Fosetyl Al was less effective against *Phytophthora* and some other diseases caused by Peronosporales. Similarly, Boughalleb *et al*. (2006) in Tunisia reported that Metalaxyl and Fosetyl Al were only effective in inhibiting mycelial growth *in vitro* at high concentrations. Additionally, Wagner *et al*. (2007), in California reported that *in vitro* inhibition of *Phytophthora* mycelial growth by Metalaxyl can be achieved with a concentration range of

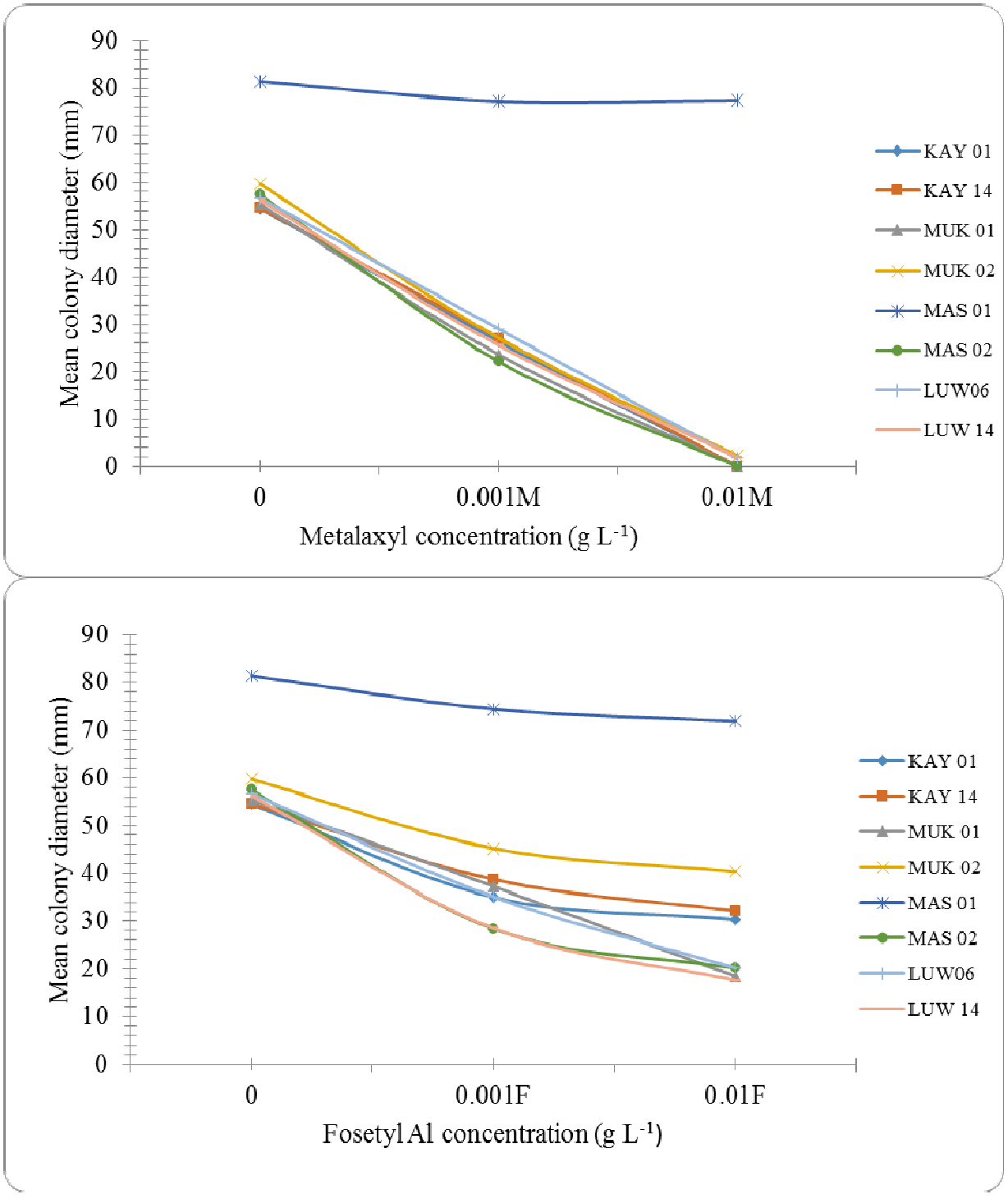


**Figure 2.** Mean colony diameter of eight representative *Phytophthora nicotianae* isolates in different Metalaxyl and Fosetyl Al concentrations.

0.001-<1g $L^{-1}$. In India, a study by Padmaja *et al*. (2015) revealed that Metalaxyl (2.5g $L^{-1}$) reduced *in vitro* growth of *Phytophthora nicotianae* by 92.9 %. However, this study has shown that at 0.1g $L^{-1}$, *Phytophthora nicotianae* growth was totally inhibited. The low inhibition of mycelia by Fosetyl Al at low concentration in this study is in agreement with Rohrbach and Schenke, (1985) who reported that high *in vitro* inhibition of mycelial growth of

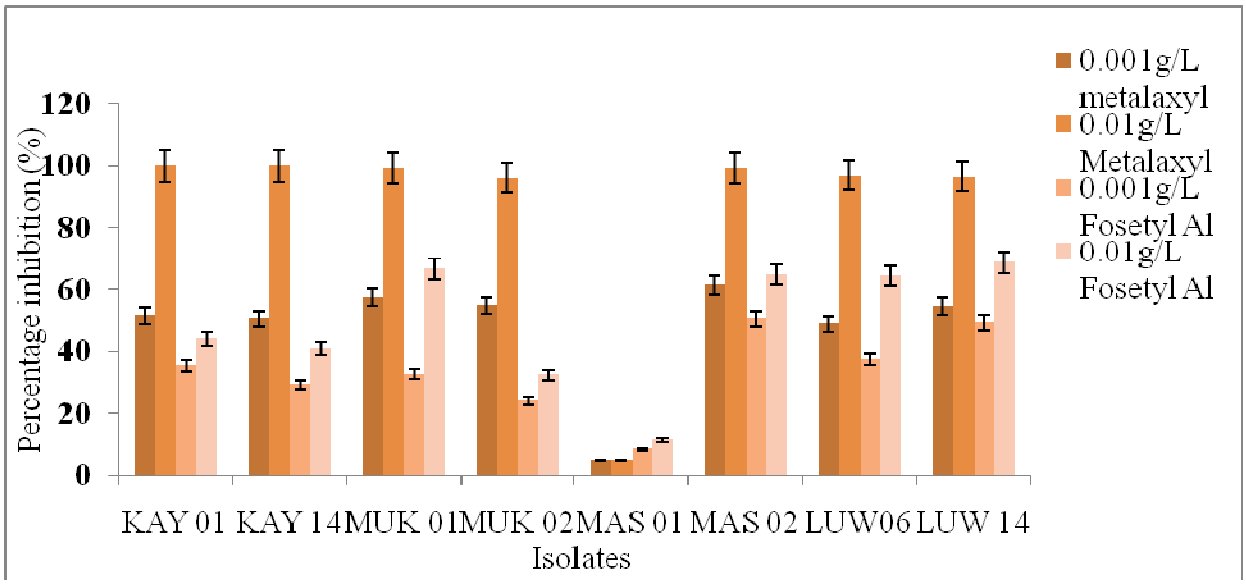


**Figure 3**. Percentage inhibition of eight representative *Phytophthora nicotianae* isolates at different Fosetyl Al concentrations.

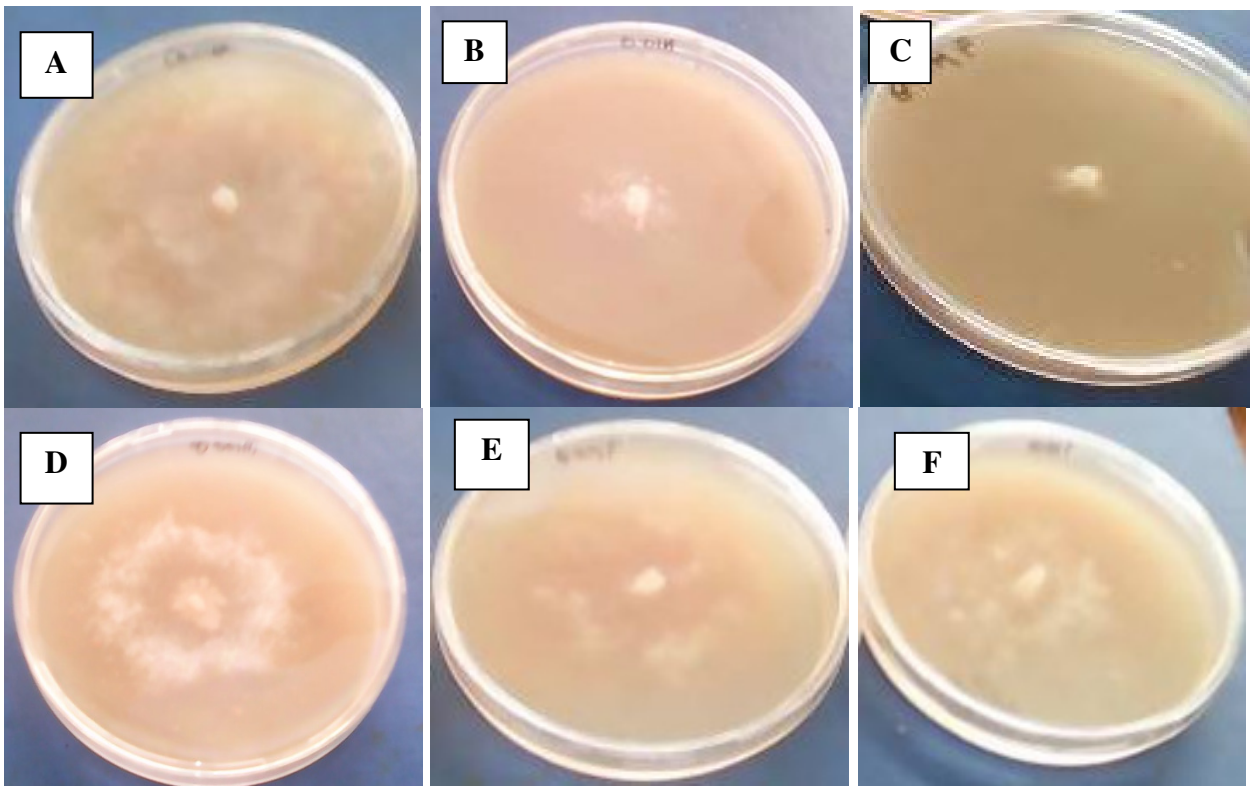


**Figure 4.** Mycelial growth of representative *Phytophthora nicotianae* isolates in different concentrations of Metalaxyl and Fosetyl Al. **A)** Control plate. **B** and **C)** Mycelial growth in petri plate amended with 0.01g $L^{-1}$ Metalaxyl concentration. **D)** Mycelial growth petri plate amended with 0.001 g $L^{-1}$ Metalaxyl. **E**) Mycelial growth in the petri plate amended with 0.01 g $L^{-1}$ Fosetyl Al. **F**) Mycelial growth in the petri plate amended with 0.001 g $L^{-1}$ Fosetyl Al.

*P. nicotianae* by Fosetyl Al can be achieved with concentrations between 0.3-1g $L^{-1}$. Similarly, Fenn and Coffey (1984) in California reported that complete mycelial growth inhibition of *Phytophthora* species using

Fosetyl Al requires the use of high concentrations. In general, the result of this study has an implication on the management of pineapple heart rot disease in Uganda. In fact, this result shows that fungicides can be incorporated in integrated management of pineapple heart rot disease in Uganda.

## Conclusions

Metalaxyl and Victory 72 powder effectively suppressed the growth of *P. nicotiane in vitro*. However, there is need to test the efficacy of these fungicides under field conditions before it can be recommended for large scale adoption for pineapple heart rot disease control and management under the Ugandan condition.

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