

In vitro response of *Phomopsis theae* to the products of *Azadirachta indica* and extracts of *Warburgia ugandensis*

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Manuscript received: 30 May 2017. Revision accepted: 11 September 2017.

Abstract. Linner CS, Birgen JK, Maingi J. 2017. In vitro response of *Phomopsis theae* to the products of *Azadirachta indica* and extracts of *Warburgia ugandensis*. *Biotechnologi* 14: 37-46. This study was done to determine the use of plant products and extracts to manage the disease caused by *Phomopsis theae*. Among the products used were Nimbecidine and Trilogy, which are products of the neem tree. The *Warburgia ugandensis* extracts from the bark, root and leaf were also used *in vitro* to determine the inhibition of *Phomopsis theae*, the causative agent of Branch and Collar Canker. These were compared with the inhibition of standard fungicides (Topsin and Saaf). Nimbecidine and Trilogy were tested at the concentration of 10 ppm, 25 ppm, 50 ppm and 100 ppm. Additionally, *W. ugandensis* extracts were tested at the rates of 10 g/100 mL, 15 g/100 mL and 20 g/100 mL. Nimbecidine inhibited growth more than Trilogy in all the concentrations and were not significantly different from those of Topsin and Saaf. Stem bark extracts of *W. ugandensis* were also effective in inhibiting the growth of *P. theae* with inhibition of 97.64 per cent in all the rates. Root was next in inhibition with 78.8, 19.45 and 9.89 per cent in 20 g, 15 g, and 10 g respectively. The leaf extracts did not inhibit growth at any rate. In liquid media, similar results were observed. In Nimbecidine, mycelial weights were significantly lower compared to Trilogy. Stem bark extracts also had lower mycelial weights, followed by the root, and then leaf among the extracts. Nimbecidine and bark extracts of *W. ugandensis* were compared with standard fungicides, Topsin and Saaf, and the extract was comparable to the fungicides both in solid and liquid media. They were able to inhibit the growth of *P. theae*. It was concluded that Nimbecidine and the bark extracts from *W. ugandensis* are potential alternatives, or can supplement the standard fungicides in the control of *Phomopsis theae* in tea.

Keywords: *Azadirachta indica*, extracts product, in vitro, *Phomopsis theae*, *Warburgia ugandensis*

INTRODUCTION

The history of tea (*Camellia sinensis* Kuntze (L.) in Kenya can be traced back to 1903 when G.W. Caine, a European settler, introduced the first seed and planted it in Limuru near Nairobi (TBK 2010). Tea is produced by smallholder farmers and large plantation such as Brooke Bond, African Highlands and Eastern Produce Limited. The large plantations are grouped in the Kenya Tea Growers Association (K.T.G.A.) and cover about 40% of Kenya tea production. Tea is an important income producer for many countries in the world. For example, tea annually contributes about 26% of Kenya's export earnings and 4% of Gross Domestic Product (GDP) (Wachira and Ronno 2004). In 2007, Kenya exported more than 360 million kilograms of processed tea that gave income to the country Kshs. 43 billion in the form of foreign exchange (TBK 2008). Since the introduction of tea in Kenya, the plantation areas have continued to grow that it has reached 149,196 hectares in 2007 (TBK 2007). This sector offers year-round jobs to approximately 639,521 farmers in rural areas, as well as providing employment in other tea-handling processes. As a labor-intensive industry, the tea sector supports the livelihood of over 3 million people directly and indirectly (TBK 2008).

The tea growing areas of Kenya are found in the Great Rift Valley. In the East Rift is the cool Arberdare plateau

and Nyabene hill. While the West Rift is decorated by the cliffs Mau is the hill of Nandi, the highlands around Kericho, Mt. Elgon and the Kisii plateau. The slopes of this plateau are 1,500 m to 2,700 m above sea level and is where tea is planted. These areas are endowed with an ideal climate for tea growth that include tropical and volcanic soils, which are rich in nutrients give a unique taste and character on the produced tea. Rainfall ranges from 1200 mm to 2700 mm per year (TRFK 2001).

Despite its importance, tea production encounters several challenges; these include the cost of tea production (labor, fuel, and electricity), mismanagement, tea-age bushes, high overhead costs, poor farming practices, climate change and shoddy roads that impede the rapid and efficient transportation of tea to the market. Fragmentation of tea farms in the face of declining global prices and rising production costs, threaten the survival of small-scale Kenyan tea farmers (Mburu 2008). Apart from all the challenges stated previously, there are also disease problems. The main diseases in Africa are the roots of *Amillaria* (*Amillaria heimii*) and the wood rot (*Hypoxylon serpens*) (Onsando et al. 1997). Others including Branch and Collar Canker (*Phomopsis theae* (*P. theae*)), Brown Blight (*Colletotrichum coccodes*) and Gray Blight (*Pestalotiopsis theae*) are very important (Anon 1991). The losses in terms of quantity due to branch and collar canker have not been counted, even though the numbers are

large (Otieno 1998). Pathogens affect carbohydrate metabolism; they also cause rosette loss and accrued uniqueness due to excessive anthocyanin accumulation. Despite its economic impact, effective preventive measures are not available, in addition to healthy wood pruning and the application of copper fungicides to prune cutting (Ponmurugan et al. 2006). Random and extreme usage of chemical fungicides for seed and soil management have led to accrued pathogen resistance (Daghman et al. 2006). For example, benzimidazole fungicides are very effective in controlling the Gray Blight of tea, but fungicide resistant isolates have appeared in most of the cultivated areas in Kenya.

Trilogy (cleansed neem oil) is known to have antifungal activity (Moline and Lock 1993). According to Mirza et al. (2000), neem products are found to be very effective at various phytophthora infestants stages. Kazmi et al. (1995), reported that 0.1% of neem oil causes a significant decrement on growth of *Alternaria alternata* and *Aspergillus* spp. Locke (1995) reported that in the field, *A. alternata*, *Aspergillus niger* and *Fusarium oxysporum* were completely controlled by using 2-10 % of neem oil. According to Niaz and Kazmi (2005), neem oil is quite effective against *Aspergillus* spp. There was a need to check the effectiveness of neem oil on *Phomopsis theae*, as such a study has never been done before.

The objectives of this study were (i) to evaluate the effects of neem products (Trilogy and Nimbecidine) on the growth of *P. theae*; (ii) to evaluate the effects of bark, root, and leaves extract of *W. ugandensis* on the growth of *P. theae*; (iii) to compare the effects of neem products and *W. ugandensis* extract on the growth of *P. theae* with standard Topsin and Saaf fungicide.

MATERIALS AND METHODS

Study site

The study was conducted in Ainamoi Village, Kericho District, Kenya (Figure 1). The study was conducted at the laboratory of Tea Research Foundation in Kenya (TRFK) in Kericho District. Kericho District is in the valley of Lake Victoria. Its geology is characterized by volcanic and metamorphic complexes. The district receives rainfall with a low evaporation rate. Temperatures range from 10°C to 29°C and rainfall patterns are so unique that the center of tea growing receives the highest rainfall of approximately 2125 mm (TRFK 2001). The soil is slightly acidic, with a pH of 5.5 which favors the growth of tea and coffee. The soil is muddy but has been well drained and has most of the nutrients needed for plant growth.

Neem products and standard fungicides source

Nimbecidine (azidarachtin) and Trilogy (hydrophobic neem extract) were neem products. Nimbecidine (azidarachtin) was manufactured by T. Stanes and Company Limited in India, while Trilogy (hydrophobic neem extract) was manufactured by Certis Company in the USA. They were purchased from Paksons Agrochemical shop in Kericho. Standard fungicides (Saaf and Topsin) were obtained from the pesticides store in TRFK in Kericho.

Culture source and inoculum preparation

One of pathogen cultures is *Phomopsis theae*. It was acquired from the stock kept at TRFK in Kericho. *P. theae* mycelium plugs were acquired aseptically from PDA agar slants of stock cultures using a flame sterilized needle and placed to the center of PDA media in 9 cm diameter petri dish. One piece was placed on each plate and incubated at 18-22°C for 7 days. The inoculation was performed in a sterile lamina flow hood for aseptic conditions.

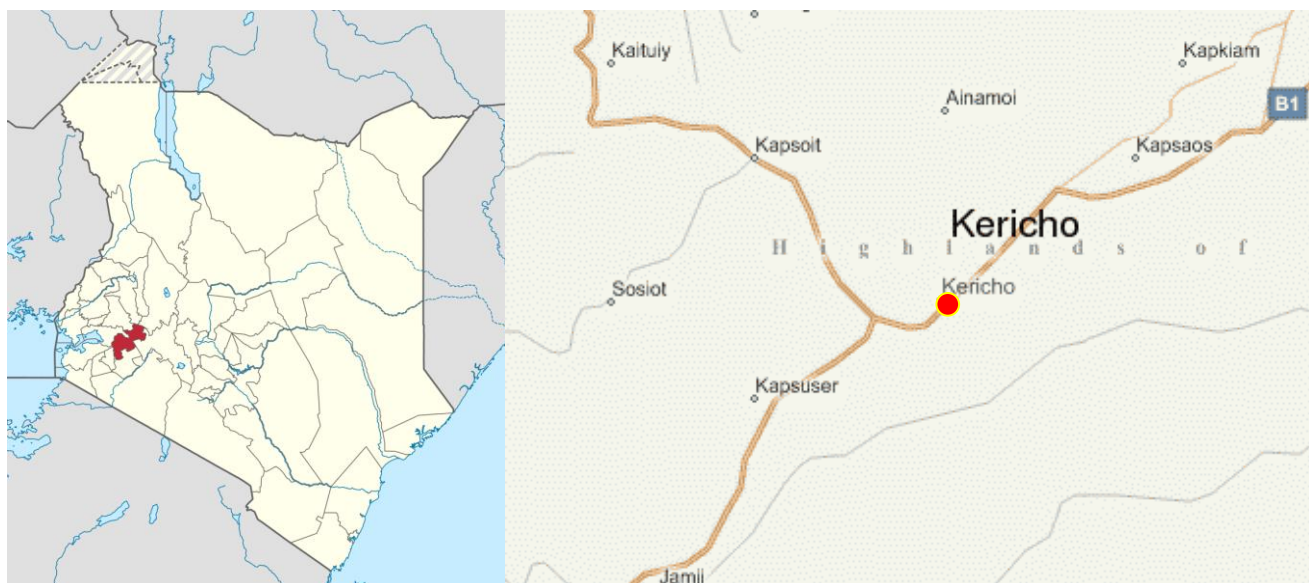


Figure 1. Map of Kenya showing location of Kericho District.

Efficacy of neem products on *Phomopsis theae* in solid media: inoculations using agar dilution method

3.90 g of PDA were liquefied in each of the seventeen 250 mL conical flasks containing 100 mL of distilled water and sterilized by autoclaving at 121°C for 20 minutes at 15 psi. The fungicides were then incorporated into the medium at the rate of 0.05 mL, 0.13 mL, 0.25 mL, and 0.50 mL for Nimbecidine and 0.53 mL, 1.33 mL, 2.67 mL, and 5.33 mL for Trilogy to make 10, 25, 50, and 100 ppm respectively.

The amended media in each Petri dish was centrally injected with 2 mm mycelia discs cut from margins of three-day old cultures of *P. theae* growing on PDA medium, using flame sterilized cork borer. The Petri dishes, which contained unamended PDA, acted as controls. The inoculated Petri dishes were arranged in a completely randomized design with four replications on a laboratory bench. Growth of the pathogen (*P. theae*) on the amended and unamended media (controls) was assessed by measuring mycelial diameter of the pathogen at three-day interval for a period of 30 days.

Efficacy of neem products on *Phomopsis theae* in liquid media: inoculation in malt extract liquid media

Eighteen grams (18 g) of malt extract were weighed one by one using analytical balance and dissolved in 1000 mL conical flasks containing 600 mL of sterilized water with 0.3 g of peptone. The neem products were incorporated into the media at 3.36 mL, 6.72 mL, 13.40 mL, and 26.80 mL for Nimbecidine and at 0.08 mL, 2.10 mL, 4.30 mL, and 8.95 mL for Trilogy and were also sterilized at 121 °C for twenty minutes at 15 psi using an autoclave. The conical flasks without the products and fungicides were set up. They were agitated thoroughly using a magnetic stirrer hot plate 400 (Gallen kamp) at 500 revolutions per minute. 150 mL of each medium were measured using a sterile measuring cylinder into 250 mL conical flasks.

A method described by Onsando (1988) to study morphology of *Armillaria mellea* was used. Mycelia from culture of *A. mellea* on MEA was used to inject petri dishes of agar. A sterile loop was used to excise 3-5 mm mycelia that were placed into the center of a Petri dish. Cultures were incubated at 25 °C for 3-6 weeks. Inoculation with the P228 isolate was carried out in a lamina flow hood. Using a sterile 2 mm diameter cork borer, mycelial agar discs were cut from the margins of colonies of three-day culture of *P. theae* isolate. Four discs were placed in each conical flask using a sterile inoculating needle. The needle was sterilized by dipping it in 70 % ethanol and flaming on a Bunsen burner. The conical flasks were incubated at room temperature on an orbital shaker (Gallen kamp SGM 300 SLUO) at 40 revolutions per minute for three weeks for the purpose of aeration. To obtain dry fungal biomass, the fungal biomass was harvested using Whatman's filter paper previously dried to constant weight. The harvested mycelium along with the filter paper were dried to constant weight and the weight of each fungal biomass determined.

In vitro* screening of stem bark, root, and leaf extracts of *Warburgia ugandensis* for their antifungal properties against *Phomopsis theae

Screening using agar diffusion method

Warburgia ugandensis samples were chopped into small pieces. Ten, fifteen and twenty grams of leaves, bark and roots were weighed separately and put into 250 mL Pyrex conical flasks which contained 150 mL de-ionized water. The samples were infused by autoclaving at 121°C for 20 minutes at 15 psi using an autoclave (Gallen Kamp). Thereafter, filtration was done, and 150 mL of the filtrate transferred into 250 mL conical flask containing 5.85 g plain agar. These were then sterilized by autoclaving at a temperature of 121°C for 20 minutes. Measurements of 20 mL of warm autoclaved agar-infusion mixture were poured into each of 9 cm diameter sterile plastic disposable Petri plates (BS 611) in a lamina flow hood. Two-millimeter diameter mycelia agar disks were cut from the margins of 7-10 days old colonies of the *P. theae* isolates cultures using a 2 mm diameter cork borer. The discs were centrally placed using a transfer needle into the 9 cm diameter plastic Petri dishes containing the leaf-agar, root-agar and stem bark-agar infusion and sealed with parafilm. Three leaf extracts, three stem bark extracts and three root extracts constitute nine treatments, with plain agar served as a control. All treatments were replicated four times in a completely randomized design and incubated at room temperature. The radial colony diameter was measured at an interval of 48 hours for a period of three weeks.

Screening in liquid media

A method described by Onsando (1987) was used. Sixty grams of fresh leaves stem barks and roots of *Warburgia ugandensis* were transferred into 1000 mL sterilized labeled Pyrex conical flasks containing 600 mL of de-ionized water. The leaves, stem bark and roots were infused at 121°C for 20 minutes at 15 psi. Using a sterile measuring cylinder, 150 mL infusion of the leaves, bark and roots were filtered aseptically with a sterilized glass funnel plugged with a small cotton wool into four 250 mL Pyrex conical flasks to make four replicates. A different funnel was used for each to avoid contamination. Inoculation, incubation, and harvesting were carried out as described in the previous experiment.

Harvesting of mycelium

Fungal mats were harvested after 3 weeks by filtering using Whatman's filter paper previously dried to constant weight and weighed together with agar blocks that formed part of the inoculums. The harvested mycelia along with the filter paper were dried in an oven (Menimen) at 60 °C to constant weight. The weight of each fungal biomass plus the filter paper were measured using an analytical balance. The dry weight of each biomass was determined.

Effect on growth of *Phomopsis theae*

Effect of neem product, standard fungicides and Warburgia ugandensis extracts on growth of Phomopsis theae using agar diffusion method

1.5 g of PDA was liquified in 100 mL of de-ionized water in 250 mL Pyrex conical flasks and sterilized by autoclaving at 121 °C for 20 minutes at 15 psi. The most active neem product (Nimbecidine) was incorporated into the medium at different rates to make 10, 25, 50, and 100 ppm. The fungicides were also incorporated into the medium at 0.05 mL, 0.13 mL, 0.25 mL, and 0.50 mL for Topsin and 0.53 mL, 1.33 mL, 2.60 mL, and 5.33 mL for Saaf to make 10, 25, 50 and 100 ppm respectively. The most effective of the *W. ugandensis* (bark) samples were chopped into small pieces, and then 10, 15 and 20 g weighed and put into 250 mL Pyrex conical flask containing 150 mL of de-ionized water. They were then infused at 121 °C for 20 minutes at 15 psi. The infusion was filtered, and 100 mL transferred into 250 mL conical flask containing 1.5 g of plain agar and autoclaved at 121 °C for 20 minutes. The agar-infusion, neem product-medium and fungicides-medium mixtures were agitated thoroughly using a magnetic stirrer hot plate, at 500 revolutions per minute. Approximately 20 mL of the mixture were poured into each of 9 cm diameter sterile disposable Petri dishes in a lamina flow hood. Inoculation, incubation, and harvesting were done as in the previous experiment.

Effect of neem product, standard fungicide and Warburgia ugandensis extract on growth of Phomopsis theae using broth dilution method

Eighteen grams of malt were weighed separately using analytical balance then dissolved in sixteen conical flasks containing 600 mL of sterilized water with 0.3 g of the peptone. The most active neem product (Nimbecidine) and fungicides (Saaf and Topsin) were incorporated as in previous section, while the most effective *W. ugandensis* extract (bark) was incorporated as in previous section. Inoculation, incubation, and harvesting were done as in the previous sections.

Data analysis

All the results were subjected to Analysis of Variance (ANOVA) using GMSTAT software to test for significant difference in radial growth and mycelial weights of *P. theae*, among the standard fungicides (Topsin and Saaf), neem products (Nimbecidine and Trilogy), *Warburgia ugandensis* extracts (leaves, bark, and roots) and control. Separation of means was carried out using Tukeys' test at $P \leq 0.05$.

RESULTS AND DISCUSSION

In vitro efficacy of neem products (Trilogy and Nimbecidine) on growth of *Phomopsis theae* in solid media

For Nimbecidine, a neem product, the radial measurements in 10 ppm, were 2.00 mm, 3.50 mm, 7.00 mm, and 12.50 mm for day 6, 15, 21 and 30 respectively.

At the concentration of 25 ppm, the measurements were 2.00 mm, 2.00 mm, 7.25 mm and 10.00 mm for day 6, 15, 21 and 30 respectively. The measurements in day 6, 15, 21 and 30 were 2.00 mm, 2.00 mm, 3.00 mm and 4.75 mm. while in 100 ppm, they were 2.00 mm for day 6, 15 and 21 and 3.56 mm in day 30 (Table 1). In Trilogy, the radial measurements were 2.00 mm, 8.73 mm, 39.50 mm, and 62.00 mm in the concentration of 10 ppm, for day 6, 15, 21 and 30 respectively. In 25 ppm, the measurements 2.00 mm, 7.25 mm, 38.25 mm, and 56.25 mm in the days of the experiment. In the concentration of 50 ppm, the measurements were 2.00 mm, 4.00 mm, 10.25 mm, and 35.00 mm in 100 ppm (Table 1).

At the lower concentration, 10 ppm, all the treatments were remarkably similar ($P \leq 0.05$, $df=79$) on day 6 and 15. However, on day 21, Trilogy was seen to differ remarkably from Nimbecidine (Table 1). At 25 ppm, no significant difference was seen on day 6 in various treatments. On day 15, there was no significant difference between Trilogy and control ($P \leq 0.05$, $df=79$), while there was remarkably different between Trilogy and Nimbecidine. On day 21, the measurements for radial growth of *P. theae* in media rectified with Nimbecidine and Trilogy were 7.25 mm and 38.25 mm, respectively. Trilogy was seen to be remarkably different from Nimbecidine treatment.

In the concentration of 50 ppm, all the treatments did not differ from each other. Similarly, on day 15, there was no significant differences ($P \leq 0.05$) among the treatments. However, on day 21 and 30 Trilogy differed from Nimbecidine treatment (Table 1). In 100 ppm, no significant differences were noted on days 6 and 15 in all the treatments ($P \leq 0.05$). On days 21 and 30, radial measurements of *P. theae* in media rectified with Trilogy were 9.00 mm and 27.00 mm. These were remarkably superior ($P \leq 0.05$, $df=79$) to that in media rectified with Nimbecidine which were 2.00 mm and 3.56 mm, respectively. In the media rectified with Nimbecidine and Trilogy, there was higher percentage of impediment compared to that of the unrectified media (control) (Figure 2, Table 1).

Impediment of fungal growth by Nimbecidine was accrued with the accrue in concentration hence rates of impediment of 95.33, 93.77, 86.6 and 83.67, at 100, 50, 25 and 10 ppm respectively were recorded. Comparatively, Trilogy also inhibited growth though in small percentages (19, 56.26, 53.60 and 49.50) unlike in Nimbecidine. Percentage impediment also accrued with accrue in the concentrations of 10 ppm, 25 ppm, 50 ppm and 100 ppm (Table 1).

Efficacy of neem products on growth of *Phomopsis theae* in liquid media

Among the neem products, higher mycelial weights were recorded in Trilogy compared to Nimbecidine. Among the various concentrations of Trilogy tested, the weights recorded were 1.47 g, 1.45 g, 1.20 g, and 0.87 g in 10 ppm, 25 ppm, 50 ppm and 100 ppm respectively (Table 2). In the media rectified with Nimbecidine, mycelial weights of 0.72 g, 0.66 g, 0.61 g, and 0.43 g in 10, 25, 50 and 100 ppm were recorded though they were not

remarkably different ($P \leq 0.05$) from each other (Table 2). At the lower concentration (10 ppm), Nimbecidine remarkably suppressed the growth of the pathogen compared to Trilogy, though the later was seen to inhibit growth more than in the control. In the concentration of 25 ppm, Trilogy was remarkably higher ($P \leq 0.05$) than those of Nimbecidine and remarkably lower than the control. In the concentration of 50 ppm of Nimbecidine and Trilogy, were 0.61 g and 1.20 g respectively (Table 2). The mycelial weights in Nimbecidine were remarkably lower ($P \leq 0.05$) than in Trilogy and in the control. At a higher concentration (100 ppm), Nimbecidine and Trilogy were remarkably different ($P \leq 0.05$) from each other. Nimbecidine inhibited growth more than Trilogy but were not remarkably different from ($P \leq 0.05$) each other but from the control. In all the concentrations, Nimbecidine was seen to remarkably suppress ($P \leq 0.05$) the growth of the pathogen compared to Trilogy (Table 2).

Efficacy of the *Warburgia ugandensis* extracts (leaf, root, and stem bark) on the growth of *Phomopsis theae* in solid media

The radial growth of *P. theae* in the media rectified with bark extracts was 2.00 mm in all the rates throughout the experimental period. However, on days 6 and 15, the radial measurements were not remarkably different from the other extracts. On days 21 and 30, radial measurements in the bark extracts were remarkably lower ($P \leq 0.05$) than that of the root and leaf extracts (Table 3). The media rectified with root extracts, also showed impediment of the growth of the pathogen.

On day 6, the radial measurements were 2.00 mm for the three rates 10 g, 15 g, and 20 g (Table 3). On day 15, radial measurements were 8.70 mm, 6.80 mm and 5.00 mm and were not remarkably different ($P \leq 0.05$) from each other. For day 21, the measurements were 33.30 mm, 28.80 mm, and 9.70 mm in 10 g, 15 g, and 20 g respectively.

There was no significant difference ($P \leq 0.05$) between the lower rates 10 g and 15 g, but were different from the

higher rate (20 g). On day 30, radial measurements were 66.00 mm, 59.00 mm and 15.00 mm, for 10 g, 15 g, and 20 g respectively.

There was significant difference ($P \leq 0.05$) among the three rates, with the higher rate being remarkably lower than the other rates. All the rates showed significant difference ($P \leq 0.05$) from that of the control. In the media

Table 1. Radial measurements and percentage impediment of *Phomopsis theae* growing on media rectified with neem products (Trilogy and Nimbecidine) on Days 6, 15, 21 and 30 after Treatment.

Treatments	Mean radial measurements of <i>Phomopsis theae</i> (mm)				% impediment	
	Number of Days					
	6	15	21	30		
Trilogy	10 ppm	2.00a ¹	8.73ab	39.50a	62.00b	19.00
	25 ppm	2.00a	7.25ab	38.25a	56.25c	56.26
	50 ppm	2.00a	4.00ab	10.25b	35.50b	53.60
	100 ppm	2.00a	4.00ab	9.00b	27.00c	49.50
Nimbecidine	10 ppm	2.00a	3.50ab	7.00bc	12.50f	83.67
	25 ppm	2.00a	2.00b	7.25cd	10.00f	86.60
	50 ppm	2.00a	2.00b	3.00c	4.75g	93.77
	100 ppm	2.00a	2.00b	2.00c	3.56g	95.33
Control		2.00a	12.50a	41.50a	76.25a	0.00

Note: ¹Mean values in the same column followed by similar letters are not remarkably different at $P \leq 0.05$

Table 2. Mycelial weights of *Phomopsis theae* in liquid media amended with neem products (Nimbecidine and Trilogy).

Treatment	Mycelial weights in grammes at different concentrations			
	Concentration (ppm)			
	10	25	50	100
Trilogy	1.47b ¹	1.45b	1.20bc	0.87cd
Nimbecidine	0.72de	0.66de	0.61de	0.43de
Control	2.16a	2.16a	2.16a	2.16a

Note: ¹Mean values in the same column followed by similar letters are not remarkably different at $P \leq 0.05$

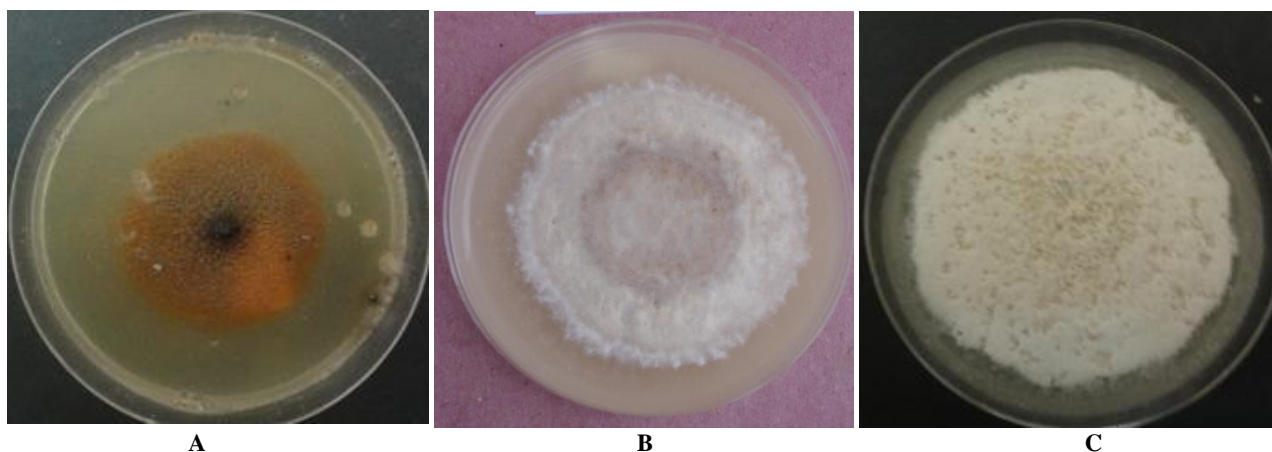


Figure 2. *Phomopsis theae* growing in media rectified with Neem products (Nimbecidine and Trilogy) and control. A. Nimbecidine, B. Trilogy, C. Control.

rectified with leaf extracts, the radial measurements on day 6 were 2.00 mm in each of the rates, 10 g, 15 g and 20. They were not remarkably different ($P \leq 0.05$) from each other. On day 15, the radial measurements were 11.30 mm, 12.50 mm and 13.00 mm in 10 g, 15 g, and 20 g respectively (Table 3). There was no significant difference ($P \leq 0.05$) among them. For day 21, radial measurements were 55.80 mm, 54.00 mm and 47.80 mm in 10 g, 15 g, and 20 g. the lower rate was remarkably different ($P \leq 0.05$) from the higher rate. All the rates were remarkably lower ($P \leq 0.05$) than that of the control. On day 30, in the three rates had radial diameter was 85.00 mm and were remarkably higher ($P \leq 0.05$) than the control.

At the lower rate (10 g), there was no significant difference among the radial measurements in all the extracts, bark, root, and leaf. On days 6 and 15, they were not remarkably different ($P \leq 0.05$) from that in the control (Table 3). On day 21, the radial measurements in the media rectified with bark extracts were significantly lower ($P \leq 0.05$) than in media rectified with root, leaf extracts and in the control. The measurements in the root extracts were not remarkably different ($P \leq 0.05$) from that of the control. The radial measurements in the leaf extracts were remarkably superior among all the extracts. On day 30, the measurements in bark extracts were also remarkably lower ($P \leq 0.05$) than in the media rectified with the other extracts and in the control. The measurements in the root extracts were remarkably lower than in the leaf and control, which were remarkably different ($P \leq 0.05$) from each other.

In the media rectified with 15 g of the extracts, there were no significant difference among the measurements in the bark, root, and leaf extracts on days 6 and 15. On day 21, the mycelial measurements in the bark were remarkably lower ($P \leq 0.05$) than in the root extracts, leaf extracts and the control. Similarly, the radial measurements were remarkably lower ($P \leq 0.05$) than in the leaf extracts and the control. The measurements in the leaf extracts were remarkably higher ($P \leq 0.05$) than in the control (Table 3). On day 30, the radial measurements in the media rectified with leaf extracts were remarkably the highest ($P \leq$

0.05) among all the treatments while the measurements in the bark were the lowest. For the highest rate (20 g) of the extracts, the radial measurements on day 6 and 12 were not remarkably different ($P \leq 0.05$) from each other and that in the control. On day 21, the measurements in the root were remarkably lower ($P \leq 0.05$) than those in the leaf and control, and higher than the measurements in the bark extracts. On day 30, the measurements in the bark extracts were remarkably the highest among the extracts and control. The measurements in the leaf extracts were significant lower ($P \leq 0.05$), even compared to the control (Figure 3). The measurements in the media rectified with root extracts were remarkably lower than in the control.

Efficacy of *Warburgia ugandensis* extracts (leaf, root, and bark) on growth of *Phomopsis theae* in liquid media

In the media amended with bark extracts, there was complete impediment of growth of *P. theae*; hence the least mycelial weights recorded. In 10 g, 15 g and 20 g of the extracts mycelial weights were 0.02 g, which was remarkably lower than that of the other extracts and the control (Table 4). The root extracts also inhibited the pathogen to certain degree. The mycelial weights in 10 g, 15 g and 20 g were 0.95 g, 0.92 g, and 0.28 g respectively. The higher rate was remarkably different from that of 10 g and 15 g, and was not different from that of the bark. In the leaf extracts, mycelial weights in 10 g, 15 g and 20 g were 1.31 g, 1.19 g, and 1.0 g respectively. They were not remarkably different from each other.

The mycelial weights of *P. theae* in 10 g of the bark extracts was 0.02 g. While in the root and the leaf, it was 0.95 g and 1.34 g respectively. The mycelial weights in the root and the leaf were not remarkably different from each other. The bark differed from the root, leaf and the control. At the rate of 20 g, the mycelia weights of the pathogen were 0.02 g, 0.28 g and 1.0 g in media rectified with bark, root, and leaf extracts respectively. The mycelial weights in that of the bark and root did not differ remarkably from each other. Leaf extracts were remarkably higher than that of the root and bark extracts. Though the bark extracts had the lowest

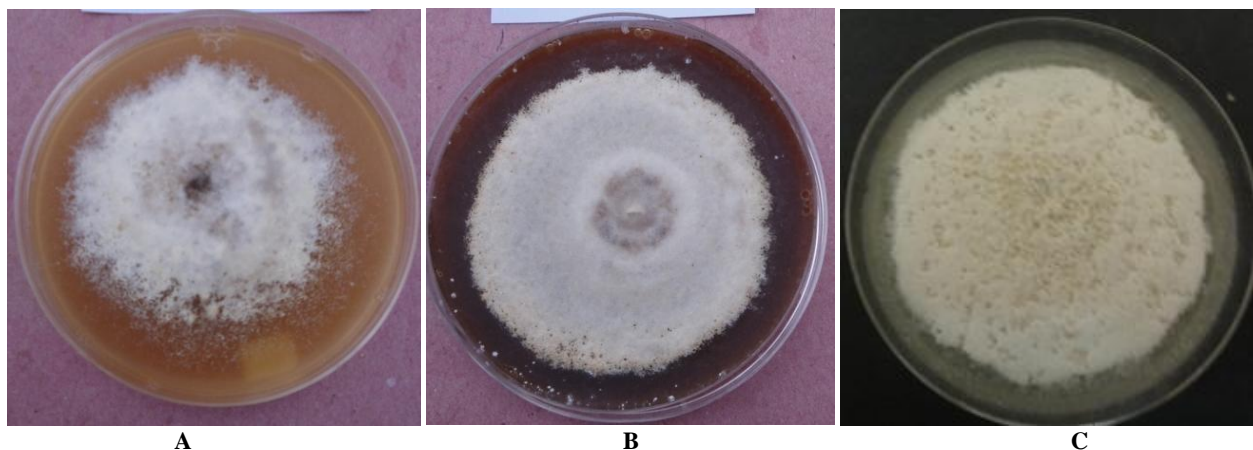


Figure 3. *Phomopsis theae* growing on media rectified with *W. ugandensis* extracts and control. A. Root extracts, B. Leaf extracts, C. Control.

mycelial weights, the lower rate did not differ remarkably from the higher rate of the root extracts. Mycelial weights of the pathogen in the media rectified with 15 g and 20 g of the leaf extracts were not remarkably different from that of 10 g of the root extracts. The unrectified media had mycelial weight of 2.31 g and was remarkably different from that of the bark, root, and leaf extracts.

In vitro efficacy of the most effective neem product (Nimbecidine), standard fungicides (Topsin and Saaf) and the most effective *Warburgia ugandensis* extracts (bark) on growth of *Phomopsis theae*

In the media rectified with standard fungicides, Topsin and Saaf, the mean radial measurements of *P. theae* were 2.00 mm in all the concentrations (10 ppm, 25 ppm, 50 ppm, 100 ppm) throughout the experimental days (6, 12, 24 and 30). These measurements were like those of the media rectified with bark extracts of *W. ugandensis*. The percentage impediment of the growth was 97.61 for Topsin, Saaf and bark extracts (Table 5).

Growth of *P. theae* in Nimbecidine was remarkably higher than in fungicides and bark extracts on days 12, 24 and 30, but was lower than in the control. The growth of the fungus in the higher concentration (50 ppm and 100 ppm), was remarkably lower than in the lower concentrations (10 ppm and 25 ppm) from day 12 to day 30 and lower than the growth in the control (Table 5). Growth in the control was the highest throughout the experiment, while in Topsin, Saaf and bark extracts, it was the lowest.

The impeding of growth impediment was highest in Topsin, Saaf and bark extracts but not different in percentage values. They were all inhibited at 97.61%. In Nimbecidine, growth impediment varied with the concentration, with the highest concentration impeding the growth at 94.33%, followed by 89.25% at 50 ppm, 85.67% at 25 ppm and 81.50% at 10 ppm. However, there was 0% impediment in the control (Table 5).

Table 3. Radial measurements of *Phomopsis theae* growing on media rectified with leaf root and bark extracts of *Warburgia ugandensis* on days 6, 15, 21 and 30.

Treatment (g)		Mean radial mycelial measurements of <i>Phomopsis theae</i> (mm)				% impediment
		Number of days				
		6	15	21	30	
Bark	10	2.0a ¹	2.0a	2.0f	2.0f	97.27
	15	2.0a	2.0a	2.0f	2.0f	97.27
	20	2.0a	2.0a	2.0f	2.0f	97.27
Root	10	2.0a	6.8a	33.3cd	66.0c	9.96
	15	2.0a	8.7a	28.8d	59.0d	19.50
	20	2.0a	5.0a	9.7e	15.5e	78.85
Leaf	10	2.0a	11.3a	55.8a	85.0a	-15.96
	15	2.0a	12.5a	47.8b	85.0a	-15.96
	20	2.0a	13.0a	54.0a	85.0a	-15.96
Control		2.0a	12.5a	39.3c	73.3b	0

Note: ¹Mean values in the same column followed by similar letters are not remarkably different at P≤0.05. g-grams per 150 mL of water

Table 4. Mycelial weights of *P. theae* in liquid media amended with *Warburgia ugandensis* extracts, from the bark, root, and leaf at different rates.

Treatment	Mycelial weights in grammes in different rates of extracts		
	Rates (g)		
	10	15	20
Bark	0.02d1	0.02d	0.02d
Root	0.95c	0.92c	0.28d
Leaf	1.34b	1.19bc	1.0bc
Control	2.31a	2.31a	2.31a

Note: ¹Mean values in the same column followed by similar letters are not remarkably different at P≤0.05. g-Grams per 150 mL of water

Table 5. Radial measurements of *Phomopsis theae* growing on media amended with standard fungicides (Topsin and Saaf), neem product, Nimbecidine, and *Warburgia ugandensis* Extract, (bark) on days 6, 12, 24 and 30.

Treatment		Means radial measurements of <i>Phomopsis theae</i> (mm)				% impediment
		Number of Days				
		6	12	24	30	
Topsin	10 ppm	2b ¹	2c	2d	2d	97.61
	25 ppm	2b	2c	2d	2d	97.61
	50 ppm	2b	2c	2d	2d	97.61
	100 ppm	2b	2c	2d	2d	97.61
Saaf	10 ppm	2b	2c	2d	2d	97.61
	25 ppm	2b	2c	2d	2d	97.61
	50 ppm	2b	2c	2d	2d	97.61
	100 ppm	2b	2c	2d	2d	97.61
Nimbecidine	10 ppm	2b	7.25b	12.75b	15.5b	81.50
	25 ppm	2b	6b	10b	12b	85.67
	50 ppm	2b	3c	7.25c	9c	89.25
	100 ppm	2b	2c	4.25cd	4.75cd	94.33
Bark	10 ppm	2b	2c	2d	2d	97.61
	15 ppm	2b	2c	2d	2d	97.61
	20 ppm	2b	2c	2d	2d	97.61
Control		12.5a	29.75a	77a	83.75a	0

Note: ¹Mean values in the same column followed by similar letters are not remarkably different at P≤0.05

Table 6. Mycelial weights of *Phomopsis theae* grown in liquid media amended with neem products (Nimbecidine), standard fungicides (Topsin and Saaf) and *Warburgia ugandensis* (bark) extracts.

Treatment	Mycelial weights in grammes at different concentrations after three weeks of incubation			
	Concentration (ppm)			
	10	25	50	100
Topsin	0.02c ¹	0.02c	0.02c	0.02c
Saaf	0.02c	0.02c	0.02c	0.02c
Nimbecidine	0.74b	0.66b	0.62b	0.45b
Bark	0.02c	0.02c	0.02c	0.02c
Control	2.31a	2.31a	2.31a	2.31a

Note: ¹Mean values in the same column followed by similar letters are not significantly different at P≤0.05

Efficacy of the most effective neem product (Nimbecidine), standard fungicides (Topsin and Saaf) and the most effective *Warburgia ugandensis* extracts (bark) in liquid media

Topsin, Saaf and bark extracts inhibited the growth in all the concentrations. Therefore, mycelial weights of 0.02 g were recorded after being incubated for 3 weeks. There was no considerable difference among the weights in these media. For Nimbecidine, the growth was better than that in the media amended by fungicides and bark extracts. There was an accrument in impediment, with an accrument in the concentration of the neem product. However, there was no considerable difference among them. Mycelial weights were considerably lower than the ones within the control in all the concentrations. The weights within the control were considerably highest in comparison to those within the amended plates (Table 6).

Discussion

Neem products efficacy (Nimbecidine, Trilogy) on Phomopsis theae growth

The observation found out that Nimbecidine drastically inhibited the growth of *P. theae*, and it was greater than the alternative neem product (Trilogy), both in solid and liquid media (table 1 and 2). Nimbecidine comprises compounds that have antifungal activity. These compounds might contain azadirachtin, melianthrol, slandin and nimbin. Dubey and Kumar (2009) pronounced that the fungicidal impact of azadirachtin was like the impact made by the fungicides bavistin and mancozeb. It has been shown to have considerably inhibited the growth of plant pathogenic fungi such as of *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani* and *Sclerotinia sclerotiorum* (Moslem 2009). The same consequences were observed by Moline and Locke (1993), who found out that neem oil had various fungicidal interests toward apple decaying fungi such as *Botrytis cinera* ex. Fr. (grey mold) and *Glomerella cingulata* (anthracnose fungus). Moreover, Bohra et al. (2006) suggested that neem has active elements including azadirachtin, nimbin and nimbinin that are antifungal in nature. Research has shown that neem oil has negative effects on *Beauveria bassiana*, by inhibiting germination, colony diameter and conidiogenesis (Hirose et al. 2000). Different results by Babu et al. (2000) stated that spraying 3 % of neem oil onto tomato pot cultures resulted in 53 % reduction in ailment incidence compared to the control, while Patil et al. (2000), found out that incidence of tomato early blight because of *Alternaria solani* was lowered by neem oil with accrued fruit yield.

Trilogy, a miticide and agricultural fungicide, showed lower impediment of growth compared to Nimbecidine (Table 1 and 2) at the higher concentration (100 ppm), it had an impediment of 64.59 per cent in solid medium that is lower than other neem product (Table 1). Similar effects were also seen in the liquid media. The results are not in agreement with that of Poilokidou (2005), who pronounced that Trilogy was powerful in opposition to *Pseudomonas xanthii* at lower concentrations in a laboratory assay. This could be due to the fact *P. xanthii* is a bacterium and therefore the difference could be caused by

the mode of action of the plant products on fungi and bacteria. The result agrees with that reported by Wszelaki et al. (2002), who found out that Trilogy had no effect on early blight and *Septoria* leaf spot disease control that are caused by *Alternaria solani* and *Septoria lycopersici* respectively. Data that is available regarding the use of Trilogy indicates that Trilogy manages diseases in cucurbits to a limited extent (Meister 1999).

In different vegetation, Trilogy affords no ailment management, an observation sister to the placement depicted by the statistics recorded from the study (Tables 1 and 2). Neem oil failed to suppress spot anthracnose on leaves of dogwood (Hagan and Akridge 2007). Seaman et al. (2004), stated that Trilogy didn't suppress foliar illnesses caused by *A. solani* and *Septoria lycopersici*. Another investigator, Aboellil (2007) mentioned that a natural product from *A. indica*, Trilogy, inhibited many growth parameters of cucumber powdery mildew pathogen (*Podosphaera xanthii*) and induced resistance in cucumber plants. The activities difference of the two neem products could be due to their active ingredients. Nimbecidine has 0.03 % azadirachtin and Trilogy, that is hydrophobic extract, has less than 0.03 % azadirachtin.

Warburgia ugandensis extracts efficacy on Phomopsis theae growth

The fungicide properties of the bark agreed with the formerly conducted research. Numerous chemical and pharmacological studies conducted to observe extracts from the bark of *Warburgia* species have showed the presence of antifungal, antiulcer, insect antifeedant, molluscal, antimycobacterial and antiheshamian active sesquiterpenes (Lunde and Kubo 2000; Wube et al. 2005; Ngure et al. 2009). Epipolygodial, mannitol, muzigadial, polygodial, tannin and warbuganal are examples of alkaloid group of metabolites present in the bark of *W. ugandensis* (Bekalo et al. 1996). A range of biological impacts consisting of trypanocidal, antiviral, fungicidal and antibacterial activity have been accredited to them.

Warburgia ugandensis extracts had been previously examined and found to show a vast spectrum antimicrobial activity against quite a few microorganisms, including *Candida utilis*. It is additionally stated to have antimicrobial activity against *F. oxysporum*, *F. solani*, *Alternaria* spp., *R. stolonifer*, *A. niger*, *R. solanacearum* and *S. ipomoeae*, which are soil pathogens in association with rotting of sweet potato and other root crops (Ristaino 1993). This suggests that the pathogens may be controlled using herbal extracts, as had also been observed in different studies (Okigbo and Nmeke 2005).

Leaf extracts were least effective in impeding the accrument of the pathogen. In the 3 rates, radial growth was not remarkably distinct from each other. The results disagreed with those of Oniango (2003), who mentioned that leaf extracts of *W. ugandensis* gave most impeding of growth of *P. theae*, even within the lowest concentration. The distinction between the observation from the contemporary study and that of Oniango (2003) could be due to the solvents used for extraction which may not have captured all the active components from the plant extracts.

Olila et al. (2001), demonstrated that this plant has both antibacterial and antifungal activities. The outcomes show that the antifungal effects in *W. ugandensis* extracts are dependent on the core and part of the plant. This agrees with work done by Olila (1993). This can be due to the difference in concentrations of the various active metabolites in the different parts of the plant.

Efficacy of the most effective neem product (Nimbecidine), standard fungicides (Topsin and Saaf) and the most effective Warburgia ugandensis extracts (bark) at the growth of P. theae

The results of the study comparing the efficacy of neem product Nimbecidine, bark extracts and fungicides (Tables 5 and 6) indicated that bark extracts inhibited the growth of *P. theae* at all the rates. They inhibited the growth even at a lower concentration. The antifungal effects of *W. ugandensis* have been previously observed. A study in Kenya showed the extracts from the plant act against soil pathogens specifically; *Fusarium oxysporum*, *Alternaria passiflorae* and *Aspergillus niger* (Ruggutt et al. 2006).

Inside the neem product, Nimbecidine, a revolutionary accrument in impediment of the pathogen growth was determined with accrument in the concentration (Table 5). Comparable observations have been made with recognition to impact on weight of mycelium (Table 6). Antifungal activity of this product has been validated in earlier research. The antifungal activity exhibition might be because of the presence of organic acids (propanic, butyric, malic, succinic, and tartaric) (Hirose et al. 2000). Bajan et al. (1998) also observed a reduction in the vegetative growth of *Beauveria bassiana* colonies caused by the industrial neem product. Different effects imply that neem oil protected the seeds of chickpea against the fungal sicknesses due to *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotium* (NRC 1992).

Saaf is also antifungal and was seen to impede the growth of the pathogen in all the concentrations and for the duration of the 30 days of the experiment (desk five). Its antifungal impacts are attributed to mancozeb and carbendazim which are the active elements. Results from a previous study by Baby and Mouli (2000), show that among fungicides screened, carbendazim was discovered to be the best in controlling thorny blight sickness of tea.

The usual fungicide, Topsin, inhibited growth at all the concentrations (desk 5). This confirms what other researchers said. Pathan et al. (2005) tested six fungicides against *Botryodiplodia theobromae*, the causal agent for mango gummosis. Topsin and Ridomil Gold have been found to be the most powerful in controlling the ailment beneath laboratory and field conditions. Antifungal activity of bark extracts of *W. ugandensis* was akin to that of control fungicides, Saaf and Topsin. They all inhibited the growth of *P. theae* in all concentrations and both in stable and liquid media (Tables 5 and 6). The neem product, Nimbecidine, was not as powerful as the standard fungicides and bark extracts, but it additionally inhibited a few percentages.

From the study, the neem product Nimbecidine was found to be more effective than Trilogy in impeding the

accrument of *P. theae*. Bark extracts were the most effective among *W. ugandensis* extracts. The antifungal activity of the bark extracts became measurable to that of the studied fungicides (Saaf and Topsin). Root extracts were additionally energetic, but concentration pendent. They were most impeding at high concentration. The results from this study indicate that the plant products namely the neem products (especially Nimbecidine) and bark extracts from *W. ugandensis* substantially inhibited the growth of *P. theae*. Consequently, they have the capability to substitute or supplement fungicides to be used to control *P. theae* and consequently the branch and collar canker of tea resulted from the pathogen.

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