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EVALUATION OF *MYRICA SALICIFOLIA* AND *PENTAS LONGIFLORA* EXTRACTS FOR THE CONTROL OF COFFEE LEAF RUST (*HEMILEIA VASTATRIX* BERKELEY AND BROOME) AT LWIRO, PROVINCE OF SOUTH KIVU, EASTERN OF DR. CONGO

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ABSTRACT

Coffee leaf rust caused by the fungus *Hemileia vastatrix* is present in the great lake country of Central Africa and in the eastern part of Democratic Republic of Congo. It caused several damages on the coffee production. The phytochemical screening of *Myrica salicifolia* and *Pentas longiflora* show secondary metabolites which have fungicidal activity. Experiments were conducted in vivo and in vitro on extract of plants against coffee leaf rust. The spraying of extract of *Pentas longiflora* and *Myrica salicifolia* were effective against coffee leaf rust respectively at 100 g/mL in vivo for both extract plants and 50 g/mL for field experiments. Results confirmed that plant extracts such as the extract of *Myrica salicifolia* and *Pentas longiflora* can be used as natural fungicides to control *Hemileia vastatrix* and thus reduce the dependence on the synthetic fungicides such as copper sulfate.

KEYWORD: *Coffee leaf rust; Hemileia vastatrix; Myrica salicifolia, Pentas longiflora, extracts, chemical control.*

INTRODUCTION

Coffee leaf rust, caused by *Hemileia vastatrix* Berk. & Br., a biotrophic basidiomycete fungus, is the most devastating disease affecting coffee crop (Morris, 1880; Talhinhas et al, 2017). Some factors are responsible of coffee production such as major diseases the coffee leaf rust and the coffee berry disease caused by the fungi *Hemileia vastatrix* Berkeley and Broome and *Antestiopsis orbitalis*, respectively. The damages and losses caused by coffee leaf rust are in the order of 10–20 % of the value of the production (Kushalappa and Eskes, 1989), and the damage to *Arabica coffee* is estimated to be approximately 35– 50 % of the production, depending on how crop is managed (Zambolim et al., 1999; Zambolim, 2016). According to study of Autrique et Perreaux (1989), the coffee leaf rust caused by the fungus *Hemileia vastatrix* is present in the great lake country of Central Africa and in the eastern part of Democratic Republic of Congo, on the western coast of Kivu Lake (Nsambu et al., 2014; Rubabura et al., 2015a, 2015b). The damages caused by coffee leaf rust results in the early fall of the leaves and dry branches, which in turn reduces the grain production in the next year. But it was revealed that the disease is usually less severe at elevations above 1,200 m, where the environment is less conducive for the rust. The most favorable condition for the infection of *Arabica coffee* by *Hemileia vastatrix* are humidity (>80%) and temperature (22 – 24°C) which as prevalent in tropical region (Chalfoun and Carvalho 1999; Zambolim et al. 1999; Zambolim 2009).

In DR Congo in general, there are no studies on the disease epidemiology and the chemical control of coffee leaf rust. Today, the disease is present in virtually all Arabica coffee-growing areas of Southern Kivu in DR Congo. Currently, control methods are based on improving varieties to increase plant resistance (Gatica-Arias et al., 2017) escaping the disease through planting coffee in higher altitudes and, fungicide applications. Chemical pesticides are known to have produced broad scale environmental impact worldwide and, at the same time, discovery and registration of new molecules to be used as insecticides, herbicides and fungicides have slowed significantly along the last decades, reducing the number of options for pest control by the farmers (Capucho et al., 2013; Ştefan et al., 2015; Cosoveanu et al., 2016).

Among the measures available for coffee leaf rust control, the most widely used is the application of protectant fungicides, mainly copper fungicides (Ventura et al. 2007, Capucho et al., 2013). There are 105 chemicals (protective and systemic) registered at the Pesticide Plant System (AGROFIT) available to control

coffee leaf rust (Capucho et al., 2013). Since fungicides are very expensive and cause serious environmental pollution, control strategies are today directed towards replacing the use of hazardous chemical fungicides by environmentally friendly natural products (Mamdouh and Eweis, 2007). Chemical control may be available to effectively and extensively reduce the effects of most fungal disease but field application of these chemical fungicides may not always be desirable. Excessive and improper use of these fungicides presents a danger to the health of humans, animals, and the environment.

Therefore, extensive searches for biofugicides that are environmentally safe and easily biodegradable have been carried out during the last two decades (Gnanamanickam, 2002).The investigation of plants containing natural fongicidal activities for plant protection has been identified as a desirable method of disease control (Rai and Carpinella, 2006; Seema *et al.*, 2011; Seint and Masera, 2011; Dwivsdi and Neeta, 2012). The use of plant extracts and biocontrol agents have been seen as a viable method for controlling plant diseases. The extremely high costs of fungicides to control diseases in coffee plantations, particularly leaf rust as well as the difficulties in its application, have led the COOPERA and CRSN/ Lwiro to give priority in the plant extract as the main part of an integrated global protection approach as prosed also by Bettencourt and Rodrigues Jr., (1988) and Silva et al., (2006).

The aim of this work was to investigate the antifungal activity of extracts of *Myrica salicifolia and Pentas longiflora in vitro and in vivo* on the leaf rust of *Hemileia vastatrix.*

MATERIALS AND METHODS

From September 2019 to May 2020, the experiment was conducted in a coffee experimental plantation and in semi-laboratory coffee garden located in the Centre de Recherche en Sciences Naturelles of Lwiro, Southern Kivu, DR Congo (altitude 1700 m, latitude 2º15' South, longitude 28º48' East). The annual precipitation average of the study area is 1300 mm with a maximum of 1800 mm and minimum of 800 mm. The average for temperature is annually 19, 5 °C. The soil is of volcanic origin but dominated by clay. The crop used for semi-laboratory experiment was formed by four lines with different concentrations of sprayed extract (50 mL of extract) of *Myrica salicifolia*, *Pentas longiflora*, positive control (copper sulfate) and negative control. In field plantation experiment were made at Lwiro 500 m of the Central Laboratory were crop were separated by 50X50 m distant. The experimental design included randomized blocks, with four treatments and tree replications. Each experimental plot consisted of 12 plants. The pesticide treatments were performed by spraying on the crop at the interval of one 15 days for exactly one month. Both sides of each plant in the crown projection were sprayed with 100 mL product. Every 30 and 60 days, the rust incidence was evaluated by counting the number of leaves with rust pustules (Capucho et al., 2011).

Plant materials

The source of *Myrica salicifolia and Pentas longiflora* leaves and stem back were obtained near the Centre de Recherche en Sciences Naturelles of Lwiro and in the National Park of Kahuzi Biega, Southern Kivu, DRCongo.

Preparation of extracts

The collected plant parts (leaves and stem back) of *Myrica salicifolia* and *Pentas longiflora* were dried and reduce to fine powder with a mortar and pass in 250 µm screen. Powders of different plants were mixed individually with distilled water in a ratio of 1:10 (w/v), ethanol 70 % and left overnight to allow the constituents to get dissolved in the solvents, then filtered through filter papers wathman N^{o} 1. The extract obtained were poured in the Erlenmeyer flasks. A subsamples were taken for phytochemical screening and another subsamples were used for the test. The last subsamples were further diluted to different concentrations by adding distilled sterile water for further use in the experiment (Harborne, 1984).

Phytochemical screening

Chemical substance contents in *Myrica salicifolia* and in *Pentas longiflora* plants were identified as alkaloid, saponin, flavonol, terpene, steroid, glucoside, phenol, quinone, tannin and lipoid in accordance with classic methods were accomplished (Sofowora, 1982; Bruneton, 2009; Diara, 2016).

Test for alkaloids: The filtrate was carefully tested with various alkaloidal reagents such as Mayer‘s reagent, Dragondroff‘s reagent and Wagner‘s reagent. To the filtrates few drops of dilute Dragondroff‘s reagent (Potassium bismuth iodide solution) was added. An orange brown precipitate indicates presence of alkaloids were used for identification of the compounds. For Wagner‘s reagent (Iodine-potassium iodide solution) the reddish brown precipitate indicates presence of alkaloids. And for Mayer‘s reagent (Potassium mercuric iodide solution) the cream precipitate indicates presence of alkaloids (Deka et al., 2017; Diara, 2016).

Test for flavonoids: The filtrate alcohol extract was subjected to Shinda’s test by concentrated hydrochloric acid. The appearance of magenta color shows the presence of flavonoid. The presence of few drops sulfuric acid conduct the formation of yellow color precipitate indicates the presence of flavonoids. Extract was treated with 3-4 drops of ferric chloride solution and it appear a brick red precipitate shows the presence flavonoids (Sofowora, 1982; Diara, 2016).

Test for phenolic compounds: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish

black colour indicates the presence of phenols (Sofowora, 1982; Bruneton, 2009; Diara, 2016).

Test for steroids: To 2 ml of ethanolic extract were mixed with 2 drops of Lieberrman-Burchard reagents (acetic acid and sulfuric acid) shake well and wait for 5 minutes. Appearance of coloration or characteristic precipitation indicate the presence of steroids (Deka et al., 2017).

Test for terpenoids: To 2 ml of ethanolic extract were mixed with 2 drops of Hirshson reagents (trichloro-acetic acid) and wait for 5 minutes. Appearance of coloration or characteristic precipitation indicate the presence of terpenoids.

Test for tannins: To the extract added 3-4 drops of Stiasny reagent. Formation of white precipitate indicates the presence of tannins.

Test for saponin: The aqueous extract were mixed vigorously with water (Foam Test). Persistent foam observed indicate the presence of saponin. To 2 ml of extract add, 3-4 drops of sulfuric acid and 3-4 drops of Potasium dichromate 10 % and wait for 2 minutes and observe the presence of precipitation characteristic for the saponin (Sofowora, 1982; Bruneton, 2009; Diara, 2016).
Test for glycosides: The filtrate was treated with 1 ml of Fehling's A and B solutions, and heated in a boiling water bath for 5-10 min. First yellow, then brick red precipitate shows the presence of glucosides (Deka et al., 2017).

Test for quinone: Benzenic extract were mixed vigorously with 2 drops of sodium hydroxide 1 % and wait for 2 minutes. The presence of precipitation indicate the quinone (Sofowora, 1982; Bruneton, 2009; Diara, 2016).

Severity measurement of leaf rust of *Hemileia vastatrix* method
Twelve Coffee tree were selected in the plantation containing leaf rust and put in pots out of the plantation near the laboratory of Entomology of CRSN/Lwiro were experiment will be conduct. The different crops selected for experimental test for *Hemileia vastatrix* were measuring for the severity of the disease by using the leaf disc method (Eskes, 1989) at the beginning of the experiment. After spraying extract tree time the month, the severity of the disease were also evaluated 30 days after according to Silva et al., (2012). Severity measurement were also conducted for crops chosen for the experiment in vivo in the experimental field located at Lwiro.

Antifungal activity assay
Antifungal activity of aqueous extracts of *Myrica salicifolia and Pentas longiflora extract* against *Hemileia vastatrix* was carried out in vivo and in vitro. Twelve coffee trees affected by the coffee leaf rust were selected from experimental field of Lwiro and potted in bags for the experimental test. Three doses (100g/mL, 10g/mL and 1g/mL) for extract of *Myrica salicifolia* and *Pentas longiflora*, solution of 1 % of sulphate of copper (positive control) and water (negative control) were used to spray to the plants. Coffee trees for experiments in vivo were conserved in the bags outside near the Agricultural Entomology laboratory at the Centre de Recherche en Sciences Naturelles (CRSN-Lwiro). For the experiment in the field plantation, 6 blocks containing 12 crops of Coffee were selected using total randomized block technique with 2 repetitions for each extract concentration (50 g/mL) of *Myrica salicifolia* and *Pentas longiflora*; positive control and negative control. Severity of *Hemileia vastatrix* of all selected crops in the plantation were evaluated before the spraying of extract and 30 days and 60 days.

Data analysis
Analyses of variance (ANOVAs) and multiple comparison procedures were performed to test the effect of extract spraying against *Hemileia vastratrix* in both experiments for the disease severity assay.

RESULTS

Phytochemical screening
The present investigation shows the phytochemical analysis of the different extract of the plants *Myrica salicifolia* and *Pentas longiflora*. Table 1 shows the phytochemical screening of the ethanolic and aquous extracts of *Myrica salicifolia* and *Pentas longiflora*.

Table 1: Phytochemical screening of *Myrica salicifolia* and *Pentas longiflora.*

Substances	*Pentas longiflora*	*Myrica salicifolia*
Alkaloïds	+	+++
Glycosides	+++	+++
Lipoïds	++	+++
Flavonoïds	+++	+++
Phenols	+++	+++
Terpenoïds	++	+++
Steroïds	+++	++
Saponins	+++	++
Quinone	+	++
Tannoids	+++	++

Legend: +++: Strong positive reaction, ++: Positive reaction, + : Less positive reaction
The result indicates the presence of tannin, phenol, terpene, steroids, saponins, quinone, and flavonoids in the extracts of *Myrica salicifolia* and *Pentas longiflora*.

Effect of extract of *Myrica salicifolia* and *Pentas longiflora* against *Hemileia vastratrix* after 30 days and 60 days of treatment
The efficacy of aqueous extract of *Myrica salicifolia and Pentas longiflora against Hemileia vastratrix in vitro* assay *after 30 days and 60 days of treatment* are depicted in Figures 1 and 2.

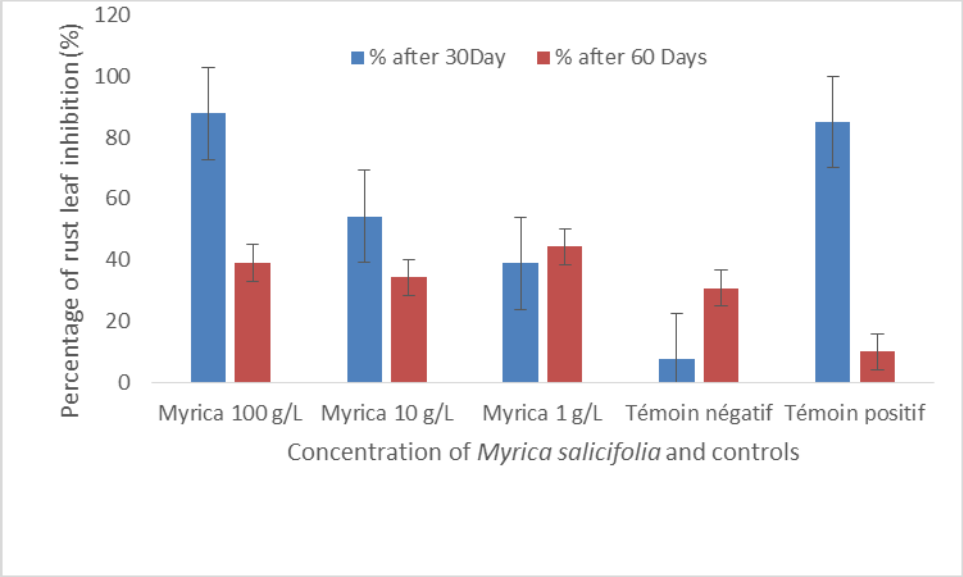

Figure 1: Percentage of rust leaf inhibition of *Myrica salicifolia* against of *Hemileia vastatrix in vitro* assay. Error bars show the standard deviation.

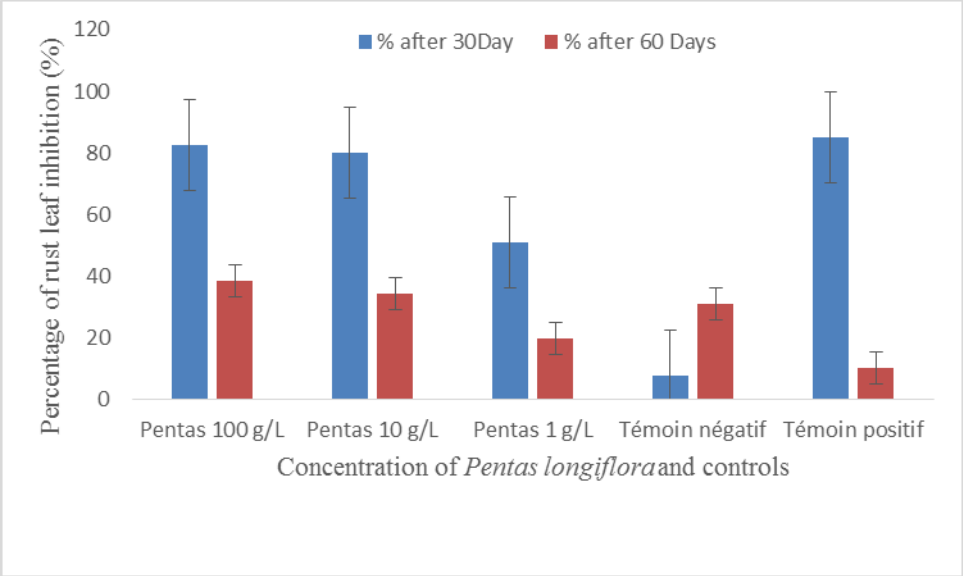

Figure 2: Percentage of rust leaf inhibition of *Pentas longiflora* against of *Hemileia vastatrix in vitro* assay. Error bars show the standard deviation.

All three treatments exhibited a reduction of percentage of rust leaf inhibition compared to negative control. In fact, *Myrica salicifolia* at 100 g/mL inhibited 87.8 % of the rust leaf of *Hemileia. vastatrix* after 30 days (t=4.18, p= 0.05) and *Pentas longiflora* at the same concentration inhibit 82.7 % (t=7.02, p=0.01). The concentration of 100 g/mL of these plants extract are comparable to the concentration of positive control (Copper sulfate of 1 %) that inhibited 85 % of rust leaf of *Hemileia. vastatrix* after 30 days. After 60 days the rust leaf of *Hemileia vastatrix* on crops are reduce and new leaf without rust appears. But, reduction of efficacy followed the concentration of extract. As concentration are reduce the efficacy of the extract in inhibited rust leaf reduce also.

The effect caused by plants extracts (100 g/mL) is very relevant considering the solution concentration of copper sulfate (1%) used actually as antifungal applying low doses to coffee control. The 1 g/mL of plants extracts of *Myrica salicifolia* assay has less compared to the same concentration of *Pentas longiflora* in both 30 and 60 days in vitro.

Experiment in coffee experimental field used to mimic the inhibition effect of rust leaf in semi-laboratory (pots), the results of the experiments are present below (Table 2).

Table 2: Severity (%) of rust leaf inhibition of *Hemileia vastatrix* on coffee in the experiment field at Lwiro.

Sample number	Negative control		*Myrica salicifolia* (50 g/mL)		*Pentas longiflora* (50 g/mL)		Positive control	
	1 Day (%)	30 Days (%)	1 Day (%)	30 Days (%)	1 Day (%)	30 Days (%)	1 Day (%)	30 Days (%)
1	80	20	90	25	80	15	50	30
2	50	50	100	15	100	25	20	10
3	25	25	100	10	95	5	80	50
4	80	50	90	15	100	7.5	30	20
5	100	70	100	10	100	5	20	10
6	100	80	100	10	100	5	50	30
Mean	73	49	97	14	96	10	42	25
STD	30	24	5	6	8	8	23	15

*Experiment were done on 12 coffees plants

Severity of coffee plants were done the first day of assay and at the 30 days after spraying 50 g/mL of different extract of *Myrica salicifolia*, *Pentas longiflora*, copper sulfate (positive control) and water (negative control).Inhibition of rust leaf of *Hemileia vastatrix* is high from extract of *Pentas longiflora* then for *Myrica salicifolia* in the experimental field. But damage on leaves after 30 days for the two extract from *Myrica salicifolia* and *Pentas longiflora* is no significant differences but significant with the controls (t=2.797, p=0.068).

DISCUSSION

Several researchers have investigated alternative control methods such as biological control, chemical substances and use of plant extracts (Carvalho et al., 2012; Carré-Missio et al., 2012; Lopes et al., 2013). The use of chemical control of coffee leaf rust is based on the spray of fungicides on the foliage (Zambolim et al., 2005b; Silva-Acuña et al., 1993a, b). Among fungicides, copper-based are the most effective (Zambolim et al., 2005b). The uses of plant extracts as diseases control agents have been studied to develop environment-friendly alternatives synthetic fungicides for the control of fungal plant diseases (Dwivedi and Neeta, 2012).

Myrica salicifolia and *Pentas longiflora* are known as medecinal plants with high concentration of phytochemical compounds. In this study, we investigated their antifungal activities against coffee leaf rust of *Hemileia vastatrix*. Our results clearly show that *Myrica salicifolia and Pentas longiflora* plant at all tested concentration had antifungal activity against the most coffee leaf rust of *Hemileia vastatrix* foliar disease pathogens under this investigation. Antifungal activities of the tested extracts were increase by increase of concentration. Extracts of *Myrica salicifolia* and *Pentas longiflora* concentrations (100 g/mL for laboratory experiment and 50 g/mL for field experiment) were the most effective at tested pathogens.

Our results are consistent with those obtained by other investigators who found an antifungal activity of Moringa plant extracts against several pathogens (Seint and Masara, 2011). Also, Mochiah et al. (2011) reported that, the presence of secondary metabolite is important in plant for fighting against parasites, fungus and bacteria which might attack vegetables production. The phenols and tannin have fungicide righteousness against plants fungicides diseases.

Our results confirmed that plant extracts such as the extract of *Myrica salicifolia* and *Pentas longiflora* can be used as natural fungicides to control *Hemileia vastatrix* and thus reduce the dependence on the synthetic fungicides such as copper sulfate. More studies are still needed in the future to test the antifungal activities of *Myrica salicifolia* and *Pentas longiflora* extracts on other different fungal plant disease in *vitro* and under field conditions.

CONCLUSION

Phytochemical study of *Myrica salicifolia* and *Pentas longiflora* reveal the presence of principal secondary metabolites groups, effectively against coffee leaf rust. *Myrica salicifolia* and *Pentas longiflora* used in our study are fluently traditional use, must be used to fight against coffee leaf rust. The fractionation of those extracts could allow probably isolating actives principles responsible of biologics activities in which coffee smallholder famers of South Kivu province could use.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BM and RK designed, performed the experiment and analyzed the data. LAC, ML and ML had given technical

guidance during the experiment, drafted and revised the manuscript. All authors read and approved the final manuscript.

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