European Journal of Academic Essays 2(3): 60-68, 2015
ISSN (online): 2183-1904
ISSN (print): 2183-3818
www.euroessays.org

IN VITRO and *IN VIVO* Botanical Control of *Rigidoporus microporus* (SW.) Overeem of Para Rubber in Nigeria

Ogbebor, O. Nicholas[1]*; Adekunle, T. Adefunke[2]; Eghafona, O. Nosakhare[3] and Ogboghodo, A. Ikponmwosa.[4]

[1]Plant Protection Division, Rubber Research Institute of Nigeria, P.M.B. 1049, Iyanomo, Benin City, Nigeria.
Email: *Ogbeb06@gmail.com*

[2] Department of Crop Science, Faculty of Agriculture,University of Benin, PMB 1154, Benin City, Nigeria.
Email: *adefunke2@yahoo.com*

[3]Department of Microbiology, Faculty of life Science, University of Benin, PMB 1154, Benin City, Nigeria.
Email: *profeghafuna@yahoo.com*

[4]Department of Soil Science, Faculty of Agriculture, University of Benin, PMB 1154, Benin City, Nigeria.
email; *iaogboghodo@yahoo.com*

Abstract: *Rigidoporus microporus* is the pathogen most feared by planters throughout the rubber tree growing regions of the world. The study was conducted to assess the effectiveness of botanical control of *R. microporus* on Para rubber in the rain forest zone of Nigeria. Twenty five plant species selected from sixteen families were screened for their fungicidal effects towards *R. microporus*, and five plant extracts exhibiting the highest mycelial inhibition were selected and assessed for concentration effects. Artificially inoculated rubber seedlings were treated with plant extracts. Significant differences were observed between the autoclaved and non-autoclaved extracts ($p < 0.05$). *Allium sativum* exhibited the highest inhibitory effect among the 25 plant extracts tested. Autoclaved extract treatments exhibited higher inhibitory efficacy on *R. microporus* compared to the non-autoclaved extracts. Extract of *A. sativum* were effective at all concentration used both in the autoclaved and non-autoclaved extracts treatments, and their effects were not significantly different at both 25% and 50% extract concentration ($P<0.05$). The percentage of plant death and presence of rhizomorph recorded at two months after inoculation for early stage of infection were higher than that recorded at the termination of five months experimental period. *Thonningia sanquinea* treatment resulted with the lowest plant death rate two months after inoculation.

Keywords: *Hevea brasiliensis*, plant extracts, *Rigidoporus microporus*, Management.

Corresponding Author: Ogbebor, O. Nicholas, Plant Protection Division, Rubber Research Institute of Nigeria, P.M.B. 1049, Iyanomo, Benin City, Nigeria, *Ogbeb06@gmail.com*, +2348055338839

Introduction

Hevea brasiliensis (Willd. ex A.Juss.) Muell. Arg. is the major economic source of latex due to its singular ability to renew its bark thus permitting a sustained latex harvest [1]. The healthy existence of the natural rubber tree is significant to the productivity of the crop [2], [3]. *Rigidoporus microporus* (Sw.) Overeem commonly called white root rot is the pathogen most feared by planters throughout the rubber tree growing regions of the world except in India [4].

In Nigeria, over half of plantation is destroyed within five years by the time the external symptoms are expressed [5]. Several studies have been carried out on the diseases of rubber in Nigeria [5]-[9] with only a limited work done on white root rot [9]-[14].

In the management of *R. microporus* the initial approach is disease avoidance; and in situations where it fails, farmers resort to the use of chemical control. The fungicides recommended for treatment are expensive (especially for local farmers who form the bulk of rubber producers in Nigeria), highly toxic to users and the environment [4]. The excessive and uncritical use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Recently, in different parts of the world, attention has been focused towards use of higher plant products as novel chemotherapeutants in plant protection. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. Intensive efforts are exerted throughout the world to elaborate control procedures that can replace chemical control of plant diseases. Numerous examples of the useful application of plant extracts in the management of plant disease had been demonstrated [6], [7], [10], [15]-[18]. The management of *R. microporus* may be achieved using improvement in traditional methods which can be achieved with knowledge of useful plants for botanical control. Controlling plant diseases using botanical means could be important in ameliorating high cost, environmental concerns and health hazards. The objective of this research was to assess *in vitro* and *in vivo* effectiveness of plant extracts against *R. microporus.*

Materials and Methods

The study was carried out at Iyanomo, Benin City in Edo State (6^0 09'23''N; $5^0$35'28''E). *Rigidoporus microporus* isolate (isolate RRIN) used in the study was obtained from infected rubber roots collected from rubber plantation and stored on Potato Dextrose Agar (PDA).

Screening of 25 Plant extracts

Twenty five plant species selected from 16 families (Balanophoraceae, Smilacaceae, Piperaceae, Solanaceae, lamiaceae, Cucurbitaceae, Rubiaceae, Moraceae, Anacardiaceae, Crassulaceae, Nyctaginaceae, Meliaceae, Amaranthaceae, Liliaceae, Asteraceae, Euphorbiaceae) were selected following suggestions supplied by local farmers and from ethno botany literature [19]. Fresh leaves free from deformities and discolouration were collected and surface sterilised [20], drier at a low temperature (28 ^{o}C) for 1h to remove excess water from the leaf surfaces. The extracts were prepared according to Ogbebor *et al*. [7] grinding in distilled water (100g leave with 100ml of water) after which the ground leaf materials were squeezed in cheese cloth. Both autoclaved and non-autoclaved extracts were amended to PDA. For the autoclaved extract the extract amended PDA were prepared by dissolving 3.9g of commercial PDA in some quantity of the filtered extract in a beaker and then made up to 100ml with more of the extract. This was dispensed into 250ml conical flasks, the mouth plugged with cotton wool, wrapped with aluminium foil and sterilized in the autoclave at 121 ^{o}C for 15 min. For the Non-autoclaved extracts the raw extracts were incorporated into the PDA after autoclaving. The medium for the control experiment was PDA alone.

Four Petri dishes per extracts amended to PDA were inoculated at the centre with a mycelial disc of 5mm in diameter taken from the periphery of actively growing 3-day-old *R. microporus* culture. The control plate was devoid of plant extract. Petri dishes were incubated at 28±2°C. Colony diameter was measured until the mycelial touched the edge of the plate 42.5mm away from the edge of the inoculum. The Percent Inhibition (PI) of growth in each of the treatment was compared to the control [6], [7], [21] with the following formula:-

$$\mathbf{PI} = \frac{100(C-T)}{C}$$

Where;

PI = Percent Inhibition of measurement of mycelial with respect to control
C = Growth in control
T = Growth in treatment.

The PI was rated for their inhibitory effects using the scale; 0% inhibition: not effective, > 0-20%: slightly effective, > 20-50%; moderately effective, >50-<100%: effective, and 100% inhibition: highly effective [14].

Concentration effect of selected plant extracts

The five most effective plant extracts from the 25 plants screened were selected for determination of concentration effects. The five plant extracts assessed were: *Ageratum conyzoides, Allium sativum, Amaranthus viridis, Ocimum*

basilicum and *Thonningia sanquinea.* For each extracts, the following concentrations were prepared according to Ogbebor *et al*. [7]: 12.5%, 25%, 50%, and 100%.

The concentration effects were assessed using the poisoned food techniques as done in the screening of 25 Plant extracts with slight modification. In this study, each plate was inoculated at the side of the Petri dish, 10mm from the edge with 3-day-old *R. microporus* culture. Radial growth measurement of the mycelia of the pathogen was taken from the edge of the inoculum to the mycelial front. Percent Inhibition of growth in each of the treatment were calculated and rated.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC), defined as the lowest concentration of extract that allows no more than 20% *R. lignosus* mycelial growth compared to the growth in the control plate was used in accessing the MIC of the extracts [22]. The autoclaved and non-autoclaved extracts were analyzed separately. The experiment was repeated and replicates from the two experiments were combined in making the assessments.

Evaluation of plant extracts on rubber tree seedlings inoculated with *R. microporus*

Seedlings of rubber tree were prepared from healthy rubber seeds in 1 m X 1 m plots, with two PVC pipes of 35cm length by 5cm diameter buried to a depth of 20cm within each plot. The open ends of the PVC pipes were covered with polytene bags held in place with rubber band to prevent soil and other materials from falling in to them. At 30 days after planting, each replicate plot was thinned to contain 40 stands of rubber seedlings of uniform growth sizes.

Rigidoporus microporus inoculum was prepared by culturing in sterilized medium (100g rubber wood sawdust, 3g rice bran and 2g sucrose, moistened with 20 ml of water) in inoculation bottles and incubated at 28-30^{o}C for 30 days. Base carrier (100g rubber wood sawdust and 3g rice bran, moistened with 20 ml of plant extracts at 100 % extract concentration) were used as carrier for the plant extracts. Treatments of seedlings were carried out by removing the pipes and filling them with the different treatments (*T. sanquinea + R. microporus*, *A. sativum + R. microporus*, *R. microporus* and control). One hundred and ten gram (110 g) each of *R. microporus* inoculum and the base carrier were applied close to the taproot of the one month old seedling at a depth of 20cm in the soil through pre-bored holes protected with PVC pipes inserted during planting of seedlings to avoid wounding of the roots. Early stage of infection was evaluated by chosen ten plants at random, excavated two months after inoculation. At the termination of the experiment (five months after inoculation) the surviving plants were removed from the soil and examined.

In order to quantify the rate of infection of each inoculated plant dead or living, several criteria were used; time between inoculation and plant death, presence of rhizomorphs on roots, length of stem, length of tap root, occurrence of reactional rhizogenesis i.e. neo-formation of lateral roots that replaced the original decayed tap root, colonization rate of root tissues or root penetration and foliar symptom. Foliar symptom was assessed by using the disease score – rating chart from which infection indices were calculated according to Adekunle and Ogbebor [6].

The *in vitro* experiment was conducted in a Completely Randomized Design with four replications; while the *in vivo* experiment was conducted in a Randomized Complete Block Design with four replications. The experiments were carried out twice and data from the two experiments were combined for statistical analysis using Genstat (8.1) software.

Results

Screening of twenty five plants extracts

The Percentage Inhibition (PI) of *Rigidoporus microporus* by twenty five different autoclaved and non-autoclaved plant extracts at 100% extracts concentration are summarized in Table 1. Significant differences were observed between the autoclaved and non-autoclaved extracts with the autoclaved extracts been most effective.

The five most effective plant extracts at 100% extract concentration from the autoclaved and non-autoclaved extracts of *T. sanquinea* (PI 99.70 % and 73.24 %) and *A. sativum* (PI 62.06 %and 62.94 %); and from the autoclaved extracts of *O. basilicum* (PI 65.88 %), *Ageratum conyzoides* (PI 56.91%), and *Amaranthus spinosus* (PI 56.18%) effectively inhibited *R. microporus* colony *in vitro*. The effect of the extract of *T. sanquinea* on *R. microporus* was significantly different from the others ($P > 0.05$).

The non-autoclaved treatments were non-effective in the inhibition of *R. microporus* with the exception of *T. sanquinea* (PI 73.24), *A. sativum* (PI 62.94) and *M. scaber* (PI 15.88). The autoclaved extracts were more effective when compared to the non-autoclaved extracts.

Concentration effect of five selected plant extracts on *R. microporus*

The concentration of plant extracts showed a significant effect on the *in vitro* growth of mycelium of *R. microporus*. Percentage inhibition by extract of *A. sativum* was highest at all levels of concentrations compared to the other extracts (Table 2).

Effective inhibitions were observed from autoclaved extracts of *A. sativum* at all concentrations; *O. basilicum* at 100% concentration; *T. sanquinea* at 50% and 100% concentrations. In the non- autoclaved extracts; effective inhibition was only observed with *A. sativum* at 50% and 100% concentrations.

Table 1. Percentage Inhibition from rapid screening of 25 plant extracts at 100% extract concentration at day 4 after inoculation.

Extract	Percentage Inhibition at day-3 (%)	
	Autoclaved	Non-Autoclaved
Acalyphya hispida Linn.	36.91^{g}	0.00^{a}
Acalyphya wilkesiana Muell Arg	41.62^{h}	0.00^{a}
Ageratum conyzoides Linn.	56.91^{i}	0.00^{a}
Allium sativum Linn.	62.06^{j}	62.94^{j}
Amaranthus spinosus Linn.	44.41^{h}	0.00^{a}
Amaranthus viridis Linn.	56.18^{i}	0.00^{a}
Azadirachta indica A. Juss.	0.00^{a}	0.00^{a}
Boerhaavia diffusa Linn.	0.00^{a}	0.00^{a}
Bryophyllum pinnatum (Lam.) Kurz.	0.00^{a}	0.00^{a}
Carica papaya Linn.	8.24^{b}	0.00^{a}
Chromolaena odorata (L.) R. M. King & Robinson	22.50^{d}	0.00^{a}
Euphorbia hirta Linn.	33.38^{fg}	0.00^{a}
Ficus elegans (Miq.) Mig.	0.00^{a}	0.00^{a}
Jatropha curcas Linn.	7.94^{b}	0.00^{a}
Mangifera indica Linn.	7.06^{b}	0.00^{a}
Mitracarpus scaber Zucc.	18.97^{cd}	15.88^{c}
Momordica charantia Linn.	7.35^{b}	0.00^{a}
Ocimum basilicum L. & O. Canum Sim.	65.88^{j}	0.00^{a}
Phyllanthus amarus Schum. & Thonn.	31.62^{ef}	0.00^{a}
Physalis angulata Linn.	27.65^{e}	0.00^{a}
Piperomia pellucida (L.) H. B. & K.	0.00^{a}	0.00^{a}
Smilax anceps Willd.	0.00^{a}	0.00^{a}
Thonningia sanquinea Vahl.	99.71^{l}	73.24^{k}
Vernonia amygdalina Del.	8.38^{b}	0.00^{a}
Vernonia cinerea (Linn.) Less.	0.00^{a}	0.00^{a}

Means with the same alphabet (superscript) are not statistically different at $P < 0.05$.

Table 2. Percentage Inhibition of *Rigidoporus microporus* by extracts of the five selected plants at day 4 after inoculation.

Extract	Concentration (%)	Mycelial percentage inhibition (%)	
		Autoclaved	Non-Autoclaved
Ageratum conyzoides Linn.	12.5	25.10^{a-h}	23.50^{a-h}
	25.0	22.69^{a-g}	23.04^{a-g}
	50.0	25.68^{a-I}	20.87^{a-g}
	100.0	34.38^{e-k}	30.69^{d-j}
Allium sativum Linn.	12.5	53.99^{lm}	18.00^{a-d}
	25.0	71.51^{no}	36.92^{h-k}
	50.0	83.78^{op}	53.63^{lm}
	100.0	92.56^{p}	65.34^{mn}
Amaranthus viridis Linn.	12.5	9.02^{i-k}	9.99^{c-j}
	25.0	35.44^{g-k}	4.37^{e-k}
	50.0	35.86^{g-k}	34.83^{f-k}
	100.0	40.62^{i-l}	35.45^{g-k}
Ocimum basilicum L. & O. Canum Sim.	12.5	15.68^{ab}	11.41^{a}
	25.0	18.22^{a-d}	14.82^{ab}
	50.0	44.51^{k}	16.68^{abc}
	100.0	75.48^{no}	30.66^{d-j}
Thonningia sanquinea V ahl.	12.5	24.45^{a-h}	21.10^{a-f}
	25.0	46.09^{kl}	42.03^{jkl}
	50.0	66.36^{mn}	62.88^{mn}
	100.0	74.17^{no}	70.91^{no}

Means with the same alphabet (superscript) are not statistically different at $P < 0.05$.

Estimation of minimum inhibitory concentration

The estimation for the Minimum inhibitory concentration of autoclaved and non-autoclaved plant extract is summarized in Table 3.

Autoclaved extracts

The lowest percentage mycelial growth compared to the control was recorded at 100% extract concentration with extract of A. sativum (23.33%). This was followed by that with T. sanquinea at 100% extract concentration (33.33%). The lowest percent mycelial growth (100%) compared to the control were recorded at both extract concentrations of 12.5% and 25% in extract of O. basilicum. The other concentrations of O. basilicum and T. sanquinea; and the other plant extracts at varied extract concentrations used recorded varied percentages ranging from 33.33% to 100%.

Non-autoclaved extracts

Thonningia sanquinea at 100% extract concentration gave the lowest percentage of mycelial growth compared to the control (23.33%) and was followed by that at extract concentration of 50% (38%). Ocimum basilicum at extract concentration of 12.5% and 25% gave 100% mycelial growths respectively compared to the growth in the control.

Table 3. Estimation of minimum inhibitory concentration of Plant extracts

Extract (%)	Concentration(%)	Percentage mycelial growth (%)	
		Autoclaved	Non- autoclaved
Ageratum conyzoides Linn.	12.5	74.67	71.33
	25.0	76.00	72.33
	50.0	70.00	73.33
	100.0	70.00	72.67
Allium sativum Linn.	12.5	64.67	89.33
	25.0	33.33	62.67
	50.0	26.67	56.67
	100.0	23.33	42.67
Amaranthus viridis Linn.	12.5	55.33	73.00
	25.0	64.67	71.67
	50.0	65.67	70.67
	100.0	48.67	64.00
Ocimum basilicum L. & O. Canum Sim.	12.5	100.00	100.00
	25.0	100.00	100.00
	50.0	68.00	96.00
	100.0	34.67	93.33
Thonningia sanquinea Vahl.	12.5	78.00	100.00
	25.0	60.00	84.00
	50.0	47.33	38.00
	100.0	33.33	33.33
L.s.d		0.51	

$\alpha=0.05$

Effect of plant extracts on rubber seedlings health inoculated with *R. microporus*

Table 4 shows seedling response to the various treatments at 2 after inoculation. At two months after inoculation of rubber seedlings with *R. microporus*, *A. sativum* and *T. sanquinea* treatments resulted in increased length in stem of seedlings compared to length of stems in seedlings in *R. microporus* treatments; while length of tap root were observed to decreased in length ($p<0.05$). *Allium sativum* treatment had the highest length of stem compared to length of stem in the control experiments. The rate of infection (plant death, presence of rhizomorphs, reactional rhizogenesis, and root penetration) reduced in the plant extracts treatment with significant differences between the plants extract treatments and *R. microporus* treatment. Foliar symptom between A. *sativum* and *R. microporus* treatment was significant difference ($p>0.05$).

Table 4. *In vivo* evaluation of plant extracts on *Rigidoporus microporus* at 2 months after inoculation**.**

Treatment	LS (cm)	LTR (cm)	Rate of infection (%)				
			PD	PR	RR	RP	FS
A. sat + R. mic	40.43^{b}	26.99^{a}	3.30^{a}	100^{b}	0.11^{a}	0.00^{a}	18.78^{ab}
T. sanq + R. mic	37.86^{ab}	26.57^{a}	1.70^{a}	100^{b}	0.00^{a}	0.11^{a}	14.50^{a}
Pathogen	34.26^{a}	28.24^{a}	9.40^{b}	100^{b}	0.61^{b}	0.56^{b}	25.17^{b}
Control	46.09^{b}	38.64^{b}	1.20^{a}	16.67^{a}	0.00^{a}	0.00^{a}	13.44^{a}

Means with the same alphabet (superscript) along the same column are not statistically different at $P < 0.05$. *A. sat =Allium sativum, T. sanq = Thonningia sanquinea, R. mic = Rigidoporus microporus*, LS = length of stem, LTR = length of tap root, PD = plant death, PR = presence of rhizomorph, RR = reactional rhizogenesis, RP = root penetration, FS = foliar symptoms, all readings are mean of 10 plants after inoculation.

At five months after inoculation of rubber seedlings with *R. microporus*, *A. sativum* and *T. sanquinea* treatments resulted in increased length in stems and tap root of seedlings compared to lengths of both seedlings in *R. microporus* treatments (Table 5). Length of stem in *T. sanquinea* treatment and *R. microporus* treatment were significantly different ($p>0.05$). *Thonningia sanquinea* treatment had the highest length of stem and tap root compared to lengths in the control experiments. The rate of infection (plant death, presence of rhizomorphs, reactional rhizogenesis, root penetration and foliar symptoms) reduced in the plant extracts treatment compared to *R.* microporus treatments with significant differences observed in reactional rhizogenesis and root penetration ($p>0.05$).

Table 5. *In vivo* evaluation of plant extracts on *Rigidoporus microporus* at 5 months after inoculation.

Treatment	LS (cm)	LTR (cm)	Rate of infection (%)				
			PD	PR	RR	RP	FS
A. sat + R. mic	61.00^{ab}	48.11^{a}	2.20^{ab}	66.67^{b}	0.06^{a}	0.17^{a}	19.83^{ab}
T. sanq + R. mic	66.44^{b}	48.22^{a}	2.20^{ab}	72.22^{b}	0.06^{a}	0.17^{a}	16.22^{ab}
R. mic	51.89^{a}	46.78^{a}	6.10^{b}	94.44^{b}	0.67^{b}	0.61^{b}	23.06^{b}
Control	69.11^{b}	59.17^{b}	1.10^{a}	18.67^{a}	0.00^{a}	0.06^{a}	12.06^{a}

Means with the same alphabet (superscript) along the same column are not statistically different at $P < 0.05$. *A. sat =Allium sativum, T. sanq = Thonningia sanquinea, R. mic = Rigidoporus microporus*, LS = length of stem, LTR = length of tap root, PD = plant death, PR = presence of rhizomorph, RR = reactional rhizogenesis, RP = root penetration, FS = foliar symptoms, all readings are mean of 25 plants at 5 months after inoculation.

Discussion and Conclusion

Many researchers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides [6], [7], [15],-[18], [23]. In the study, extracts of the 25 selected plants showed varied inhibitory efficacies against *R. microporus*. *A. sativum* was the most effective in the list among the 25 plant extracts tested and was followed by *T. sanquinea*. *Allium sativum* has been shown several times to have antifungal properties against other plant pathogens [15], [18], [24], [25]. Ijato [25] reported antifungal effects of extracts of *A. sativum* and *Nicotiana tobacum* against soft rot of yam (*Dioscorea Alata*).

Autoclaved extracts exhibited higher inhibitory efficacies on *R. microporus* compared to the non-autoclaved extracts. Extracts of *A. hispida, A. wilkesiana, A. conyzoides, A. viridis, C. odorata, P. pellucida* were found to be effective in the autoclaved treatment but ranged from not effective to moderately effective in the non-autoclaved treatment. *Allium sativum* demonstrated the highest PI in the autoclaved treatment while *T. sanquinea* had the highest inhibitory efficacy in the non-autoclaved treatments. This finding is supported by earlier study by Stangarlin *et al.* [23], where autoclaved extracts (eucalyptus, neem, garlic, citronella, mint, rue, yarrow, ginger, basil, camphor, turmeric and *Ocimum*) exhibited higher inhibition of mycelial growth of some phytopathogens. In the study Stangarlin *et al.* [23]

attributed the lower inhibitory efficacies demonstrated by the non-autoclaved extract to the presence of thermo-sensible compounds which may favour the growth of the fungus.

There were significant effects of the different extracts concentrations on the inhibition of *R. microporus* in the study. Extract of *A. sativum* were effective at all concentration used both in the autoclaved and non-autoclaved extracts treatments. This study is also in line with earlier reports by Ogbebor *et al.* [18], where mycelial inhibition was significant with the higher concentrations of extracts used. D'Aulerio *et al.* [24] reported the effectiveness against some plant pathogens of aqueous extracts of garlic. The result in this study showed that extracts of the different plants species are substantially varied in their antifungal potentials. These differences are to be expected since plants vary in their chemical constituents, habitats and stages at which they were collected. Differences in the nature and concentration of inhibitory compounds even between different plants parts have been reported [26].

In the autoclaved plant extracts only extract of *T. sanquinea* at 100% extract concentration gave value (33.33%) close to the Minimum inhibitory concentration according to Savitha and Rathnavijaya [22]. Similar result was also obtained for the non-autoclaved plant extract with *T. sanquinea*. However, extract of *A. sativum* at 100% extract concentration recorded the lowest value (23.33%) which had the closest value to the minimum inhibitory concentration (20%). The results for estimation of minimum inhibitory concentration with the plant extracts therefore indicate that for the plant extracts to be much effective on *R. lignosus,* higher concentration of plant extract must be used. In this study water extraction of the active constituent from the plant was employed because the study was aimed at developing simple methods of extraction of plant extracts for the management of white root rot for poor farmer who form the bulk of rubber producers in Nigeria. With the results obtained in this study, we therefore suggest that for these plants extracts to be more effective, other more efficient and more complex method of extraction like chemical extraction would have to be employed.

The results of *in vivo* assays showed that the percentage of plant death and the presence of rhizomorphs recorded at two months after inoculation for early stage of infection were higher than that recorded at the termination of five months experimental period after inoculation. *T. sanquinea + R. microporus* treatment had the lowest plant death at two months after inoculation. All the treatments developed rhizomorphs at the surface of their tap roots with significant reduction recorded in the controls at both two months and five months after inoculations. This observation may suggest a relationship between pathogenicity and the capacity to produce rhizomorphs. Previous works have demonstrated that rhizomorphogenesis and initial penetration of *R. microporus* occur during the first two months after inoculation [27].

Several workers have worked on the effects of concentrations of extracts on different plant pathogens [18], [24], [26]. Al-Abeed [26] observed significant effects of concentration levels of aqueous extracts of varied wild plants on some plant pathogenic organisms.

Decline in mortality rate (plant death) from the third months onward suggested a reduction in fungal activity. This contributed to the reduction in presence of rhizomorphs observed at the termination of the experiment. Similar results had been described in earlier work with rubber seedlings artificially inoculated by *R. microporus* [28]. Decrease of the fungal activity was observed to be due to exhaustion of trophic reserves in the substrate of the inoculum. The length of stem and length of tap root were respectively highest in *A. sativum + R. microporus* and *T. sanquinea* treatments at both times of observations compared to the control. Percentage of plant death recorded with *T. sanquinea + R. microporus* treatment was lower than that in the control at early stage of infection.

In conclusion, extract of *A. sativum* performed better in the *in vitro* study. *Thonningia sanquinea* extract was observed to increase the health of the rubber tree seedlings when compared to extract from *A. sativum* in the *in vivo* study.

Many workers have compared the effectiveness of the use of biological control to chemical control in the management of plant pathogens [29]-[30]. Tewari [29] demonstrated lesser cost of application of *Ocimum sanctum* (RS 375/ha) compared to synthetic fungicide of Ediphenphos (RS 1430/ha) or Carbendazim (RS 1580 /ha).

The use of botanicals control may not be a panacea; however, they are useful tools for plant protection. For effective control to be achieved control measure should be applied on or before the onset of infection. However, disease prevention is often achieved with much less cost and trouble than treating the disease after it has set in. There is a large reservoir of natural fungicide in plants around us which with continued research would provide safer and effective alternative to synthetic fungicides.

References

[1] J.B. Gomez, "Anatomy of *Hevea* and its influence on latex Production," Malaysian Rubber Research and Development Board, pp. 76, 1982.

[2] N.O. Ogbebor, V.I. Omorusi, G.A. Evueh, "Evaluation of nine Hevea brasiliensis clones for mistletoe infestation and the effect on latex yield," Journal of Natural Rubber Research, 20 (1 & 2), pp. 87 – 89, 2007a.

[3] N.O. Ogbebor, "The status of three common leaf disease of Para rubber in Nigeria," Journal of Animal & Plant Sciences, 6 (1), pp. 567-570, 2010.

[4] C.K. Jayasinghe, "White root disease of the rubber tree. An overview," Proceedings of the International Workshop on white root disease of rubber," pp. 1-22, 14th – 16th, December, 2010.

[5] V.O. Otoide, "*Thonningia sanquinea* a new parasite of rubber roots," Trop. Pest Mgt, 28(2), pp. 186-188, 1978.

[6] N.O. Ogbebor, A.T. Adekunle, "Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* Muell. Arg.) using extracts of some plants," African Journal of Biotechnology, 4(9), pp. 996 – 1000, 2005.

[7] N.O. Ogbebor, A.T. Adekunle, D.A. Enobakhare, "Inhibition of *Colletotrichum gloeosporioides* (Penz) Sac. causal organism of rubber (*Hevea brasiliensis* Muell. Arg.) leaf spot using plant extracts," African Journal of Biotechnology, 6(3), pp. 213 – 218, 2007b.

[8] A.G. Evueh, J.A. Okhuoya, O.O Osemwegie, I.H. Attitalla, O.N. Ogbebor, "Evaluation of Phylloplane fungi as biological agent of Corynespora leaf fall disease of rubber (Hevea brasiliensis Muell. Arg)," World Journal of fungi and plant biology, 2 (1), pp. 01-05, 2011.

[9] N.O. Ogbebor, A.T. Adekunle, O.N. Eghafona, A.I. Ogboghodo, "*Ganoderma psuedoferreum*: Biological control possibilities with microorganisms isolated from soils of rubber plantations in Nigeria," African Journal of General Agriculture, 6 (4), pp. 301-305, 2010.

[10] A.T. Adekunle, N.O. Ogbebor, "*In vitro* control of *Colletotrichum gloeosporioides* (Penz.) Sacc. and *Rigidoporus lignosus* using biological agents,". Bioscience Research Communications, 21 (4), pp. 199 – 202, 2009.

[11] N.O. Ogbebor, A.T. Adekunle, O.N. Eghafona, A.I. Ogboghodo, "Incidence of Rigidoporus lignosus (Klotzsch) Imaz of Para rubber in Nigeria," Researcher, 5(12), 181-184, 2013.

[12] N.O. Ogbebor, V.I. Omorusi, A.T. Adekunle, "Incidence of red root disease in Hevea caused by *Ganoderma psuedoferreum*: a first report from Nigeria," Rubber Science, 26(2), pp. 304-307, 2013.

[13] N.O. Ogbebor, V.I. Omorusi, K. Orumwense, K. Ijieh, "The status of white root rot in rubber plantations in Nigeria. Journal of Plantation Crops," 42(1), pp. 142-144, 2014.

[14] N.O. Ogbebor, A.T. Adekunle, O.N. Eghafona, A.I. Ogboghodo, "Biological control of *Rigidoporus lignosus* in *Hevea brasiliensis* in Nigeria," Fungal biology, 1 1 9, pp. 1-6, 2015.

[15] P. Lakshmanan, "Effect of certain plant extracts against *Corynespora cassiicola*," Indian Journal of mycology and Plant pathology, 20 (3), pp. 267- 269, 1990.

[16] G.A. Evueh, N.O. Ogbebor, "The use of phylloplane fungi as biological control agents of Colletotrichum leaf diseases of rubber (*Hevea brasiliensis* Muell. Arg.)," African Journal of Biotechnology, 7 (15), pp. 2569 – 2572, 2008.

[17] N.O. Ogbebor, A.T. Adekunle, "Inhibition of *Drechslera heveae* (Petch) M. B. Ellis, causal organism of Bird's eye spot disease of rubber (*Hevea brasiliensis* Muell Arg.) using plant extracts," African Journal of General Agriculture. 4(1), pp. 19 – 26, 2008.

[18] N.O. Ogbebor, V.I. Omorusi, A.T. Adekunle, G.A. Evueh, "*In vitro* evaluation of two botanical fungicides against four pathogens of rubber in Nigeria," Journal of sustainable Tropical Agricultural Research. 16, pp. 86 – 91, 2005.

[19] *L.S. Gill, "Ethomedical* Uses of Plants in Nigeria," *University of Benin Press, Benin-City*, pp. 276, 1992.

[20] International Rubber Research and Development Board, "International Rubber Research and Development Board (IRRDB) Workshop on *Corynespora* leaf fall of Rubber," pp. 2, 6-9 June, 2000.

[21] C. Kaiser, R. Van der Merwe, T.F. Bekker, N. Labuschagne, "South African Avocado Growers' Association," Yearbook, 28, pp. 70-74, 2005.

[22] S. Savitha, C. Rathnavijaya, "Minimum inhibitory concentration and antioxidant properties of Andrographis paniculata using different solvent extracts," International Journal of Chemical Science and Technology. 1(1), pp 21-28, 2011.

[23] J.R. Stangarlin, O.J. Kuhn, L. Assi, K.R.F. Schwan-Estrada, "Control of plant diseases using extracts from medicinal plants and fungi. Science against microbial pathogens," Communicating current research and technological advances, pp. 1033 – 1042, 2011.

[24] A.Z. D' Aulero, A. Zambonalli, A. Banachi, I. Albanni, "Effects of essential oils on Phytopathogenic fungi In vitro," Phytopathol, 144, pp. 491 – 494, 1996.

[25] J.Y. Ijato, "Evaluation of antifungal effects of extracts of *Allium sativum* and *Nicotiana tobacum* against soft rot of yam (*Dioscorea alata*)," Researcher, 3(2), pp. 1-5, 2011.

[26] A.S. Al-Abed, "Possible antifungal effects of aqueous extracts and residues of some common wiled plant species on certain plant pathogenic fungi," M. Sc. Thesis, University of Jordan, p 81, 1992.

[27] D. Nandris, M. Nicole, J.P. Geiger, "Root rot disease of rubber," Plant Disease, 71, pp. 298–306, 1987.

[28] D. Nandris, M. Nicole, J.P. Geiger, "Inoculations of young plants of *Hevea brasiliensis* by *Rigidoporus lignosus* and *Phellinus noxius*," Eur. J. For. Pathol, 13, pp65-76, 1983.

[29] S.N. Tewari, "*Ocimum sanctum* L., a botanical fungicide for rice blast control," *Tropical Science*. 35, pp 263 – 273, 1995.

[30] D. Eksteen, J.C. Pretorius, T.D. Nieuwoudt, P.C. Zietsman, "Mycelial growth inhibition of pathogenic fungi by extracts of South African plant species," Annals. Appl. Biol. 139(2), pp 243- 249, 2001.