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Evaluation of Different Botanicals for the Control of Coffee Leaf Rust (*Hemileia vastatrix* Berkeley and Broome)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RMM and NM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors BTM and PC managed the literature searches and analysis of the study. Author CM managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Coffee is one of the major foreign currency earners for Zimbabwe, with over 95 % of the total production being exported. Production of coffee is constrained by diseases such Coffee Leaf Rust caused by the fungus *Hemileia vastatrix*. Chemical control is widely used but it is expensive and not environmentally friendly. Hence this experiment was conducted in the laboratory at Coffee Research Institute, Chipinge in November 2015, with the objective of examining the efficacy of four botanical extracts viz Lemon grass (*Cymbopogon citratus*), Aloe vera (*Aloe barbadensis*), Moringa (*Moringa oleifera*) and Tobacco (*Nicotiana tabacum*) extracts in three different concentrations (100, 50 and 25% leaf extracts) as compared with Copper oxychloride as a standard fungicide and distilled water as a negative control. The experiment was laid in a Completely Randomized Design (CRD) design with a 4 x 3 factorial structure and was replicated four times. The first factor was the above mentioned four botanicals and the second was the respective concentrations (25%, 50% and

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100%). Each experimental unit comprised three Petri dishes. Data on spore germination inhibition percentage was recorded. Results showed that the different plant extracts were effective in inhibiting germination of the spores though their effects were different. There was a strong correlation between plant extract concentration and spore germination inhibition for tobacco, Aloe vera and Moringa. Increase in plant extract concentrations resulted in a marked increase in spore germination inhibition. Of the four plant extracts; Moringa and lemon grass proved to be the most effective while Aloe vera was the least effective. It can be concluded that the four plant extracts are effective in inhibiting fungal spore germination. It is recommended that further studies be done in order to establish the active compounds which are responsible for the anti fungal activities. Also more experiments should be done to determine effects of these plant extracts on other fungal diseases which affect crops.

Keywords: Coffee leaf rust; Hemileia vastatrix; plant extracts; spore germination.

1. INTRODUCTION

Coffee Leaf Rust is a disease caused by *Hemileia vastatrix* Berkeley and Broome, and is one of the most devastating diseases of Arabica coffee in all coffee growing regions of Zimbabwe and almost all coffee growing areas worldwide [1,2]. The disease is a threat to coffee production in both smallholder and large scale farms. It has been reported to be very expensive to control the disease and this is mainly due to over dependence on synthetic fungicides and it is estimated to cost between US$1 billion and US$3 billion per year globally [3]. If the disease is left unchecked, it will lead to defoliation of coffee trees prematurely, which results in die-back and reduction in yield, quality. When infection is serious, Coffee Leaf Rust may reduce yields by up to 10-20% for a plantation and 70-80% for an individual tree [3-6].

Fungicides and host plant resistance can be effectively used in the management of Coffee Leaf Rust [7]. In Zimbabwe and other coffee growing countries, Coffee Leaf Rust is mainly controlled through the use of copper-based fungicides especially Copper Oxychloride and other triazoles such as Triadimefon (Bayleton), Triadimenol (Bayfidan) and Nativo 300SC (Tri-floxystrobulin + Tebuconazole) [8,9]. Copper based fungicides are very effective in controlling Coffee Leaf Rust, and copper has a "tonic effect" on coffee plants, which means that it leads to increase in yields independent of its effect on Coffee Leaf Rust control [1]. Despite the advantages of chemical control, it has been reported lately that copper based fungicides have a negative effect on non-targeted organisms in both terrestrial and aquatic ecosystems [1]. In Zimbabwe, fungicides such as Tridemefon are being phased due to long residual effect in the environment [10]. Use of botanicals for Coffee Leaf Rust control could be a better alternative. Plant extracts are non-polluting, cost effective, non-hazardous and do not disturb ecological balance [11,12]. Most botanicals are compatible with bio-control or relatively soft on natural enemies and are easily bio-degradable [12]. Some of the botanicals have a broad spectrum effect; some having both fungicidal and insecticidal properties. In Zimbabwe there has been very limited research on the efficacy of botanicals on the control of coffee diseases hence the need for this study to determine the efficacy of different plant extracts (*Nicotiana tabacum*, *Aloe barbadensis*, *Cymbopogon citrates* and *Moringa oleifera*) on the control of Coffee Leaf Rust.

2. MATERIALS AND METHODS

2.1 Description of Study Site and Experimental Design

The experiment was conducted at Coffee Research Institute, Chipinge, in the laboratory. The research station is located at a latitude $20^{o}12'$ South, longitude $32^{o}37'$ East and it is at an altitude of 1100 above sea level.

The experiment was laid out in a Completely Randomized Design (CRD) with a 4 x 3 factorial structure. The first factor was plant extract type which had 4 levels (Moringa, (*Moringa oleifera*), Lemon grass (*Cymbopogon citrates*), Tobacco (*Nicotiana tabacum)* and Aloe vera (*Aloe barbadensis*) while the second factor was plant extract concentration with three levels (100%, 50% and 25%). The experiment was replicated four times. A positive control (Copper oxychloride 80% WP at 1 g/litre of water) and a negative control (distilled water) were also used in the experiment. Each

treatment comprised of three Petri dishes of the same plant extract.

2.2 Preparation of the Plant Extracts

Plant extracts which were obtained from a forest at the Coffee Research Institute, were prepared by crushing fresh plant leaves using a pestle and mortar before extraction. The plant extracts were prepared by mixing 100 g of crushed leaves with 100 ml water (aqueous) and this was left to stand for 16 hours. After 16 hours the soaked extracts were then be filtered through a fine cloth and centrifuged at 600 rpm for 10 minutes to produce 100% (neat) extract. 50% leaf extract was prepared by mixing one part neat extract with one part distilled water and 25% mixed at the ratio of 1 part neat extract to 3 parts distilled water.

2.3 Preparation of the Urediniospores

A spore suspension of *Hemileia vastatrix* (8 x 10^6 spores/ ml) was prepared using sterile distilled water and spore counting was done using a haemocytometer.

2.4 Treatment Application and Data Collection

1 ml of each plant extract concentration was added to 9ml of sterile 2% water agar in a test tube and shaken well using an agitator (Vortex mixer SA3, Stuart Scientific) to ensure a thorough mix. The test solution was poured into a Petri dish and then allowed to cool and solidify. After this, 0.5 ml *H. vastatrix* urediniospores suspension was pipetted out, and spread evenly on to the medium. The inoculated Petri dishes were incubated for 16 hours under at ±25℃ in the dark room. After 16 hours one drop (0.5ml) of 0.1% Mercuric chloride was added into each Petri dish. Germinated spore counting was done by microscopic examination of 100 spores per slide using a tally counter through Stereoscopic microscope (Nikon 8771, Japan). Urediniospores with germ tubes of at least half of the length of their larger diameter were considered germinated. The values were expressed as a percentage by using the formula:

$$Percentage\ of\ germinated\ urediniospores =$$

$$100\ x\ \frac{number\ of\ urediniospores\ germinated}{number\ of\ urediniospores\ counted\ under\ the\ microscopic\ field}$$

Germination percentage of urediniospores was also used to calculate percentage inhibition. Percent inhibition due to various treatments was computed by using the Abbott correction formula as follows:

$$Percent\ spore\ germination\ inhibition =$$

$$100\ x\ \frac{Spore\ germination\ in\ control - Spore\ germination\ in\ treatment}{Spore\ germination\ in\ control}$$

2.5 Data Analysis

Data obtained from the experiment was analyzed using Analysis of variance (ANOVA) by the use of Genstat 14th Edition. Means of significant results were separated using Fishers protected Least Significance Difference test. The data for means from Genstat were used to do correlation analyses between different extracts concentrations and spore germination inhibition.

3. RESULTS

3.1 Efficacy of Different Concentrations of Plant Extracts on Percent Spore Germination Inhibition

There was an interaction (*P* = 0.022) between plant extracts type and their different concentrations on percent spore germination inhibition. There was also a strong correlation between plant extract concentration and spore germination inhibition percent. As the plant extract concentration increased this resulted in an increase in the percent germination inhibition of spores causing Coffee Leaf Rust for all the treatments. Strong positive correlations were noted for tobacco (R^2=1), Aloe vera (R^2 = 0.9968) and Moringa (R^2 = 0.9461) while a relatively weak correlation was recorded do lemon grass (R^2 = 0.6474) (Fig. 1). The 25% concentration resulted in the least percent germination inhibition across all treatments. Of the plant extracts, Moringa and Lemon grass were the most effective at 100% concentration in inhibiting spore germination while Tobacco and Aloe vera performed relatively lower though these two were not statistically different from Copper oxychloride (control). For the 50% and 25% concentrations; Lemon grass recorded the highest spore inhibition percent which was statistically different from all other plant extracts while Tobacco recorded the least. Distilled water did not inhibit Coffee Leaf Rust spore germination at all (Table 1).

Table 1. Efficacy of different plant extracts and their concentrations on spore germination inhibition

Treatment	Spore germination inhibition percent
Moringa 100%	97.30[a]
Lemon grass 100%	94.50[a]
Lemon grass 50%	93.71[ab]
Lemon grass 25%	85.35[bc]
Moringa 50%	84.24[cd]
Tobacco 100%	77.45[de]
Aloe vera 100%	75.75[ef]
Copper oxychloride @1g/L water	69.01[ef]
Moringa 25%	65.25[fg]
Aloe vera 50%	59.94[gh]
Tobacco 50%	58.25[gh]
Aloe vera 25%	51.27[h]
Tobacco 25%	0[i]
Distilled water	
P value	0.022
LSD	8.73
CV%	8.7

4. DISCUSSION

All the four plant extracts which are Aloe vera (*Aloe barbadensis*), Lemon grass (*Cymbopogon citratus*), Tobacco (*Nicotiana tabacum*) and Moringa *(Moringa oleifera*) gave varying degrees of Coffee Leaf Rust control in their respective concentrations. Plants have the capability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [12,13,14]. The results of the experiment showed that all the four plant extracts tested have some fungicidal activity and can be used in the control of Coffee Leaf Rust when compared with the standard fungicide (Copper oxychloride) and the untreated control (distilled water). Moringa at 100%, Lemon grass at 100% and lemon grass at 50% performed better than Copper oxychloride and did not significantly differ from each other. Lemon grass at 25% and Moringa at 50% also outperformed the Copper oxychloride. Tobacco at 100%, Aloe at 100%, Copper oxychloride and Moringa at 25% were not significantly different from each other. However, distilled water (control) did not inhibit Coffee Leaf Rust spore germination and the non- inhibitory effect suggests that the spores were viable. A strong correlation was observed between different concentrations of Tobacco, Aloe vera and Moringa with spore inhibition percent. This shows that these plant extracts become more effective in fungal control as their concentrations increase. Despite the fact that all the bio pesticides in all their concentrations managed to control *Hemileia vastatrix* spore germination*,* Lemon grass was more consistent. Results showed a weak correlation between lemon grass concentrations and percent spore germination inhibition. Lemon grass at 50% was not significantly different from the 100% meaning that increasing the concentration from 50% upwards had no economic advantage. The findings in this study agreed with earlier report by Gurjar et al. [12], who indicated that essential oil from Lemon grass *(C. citratus)* was found to be effective against post-harvest anthracnose of Mango fruit. Tzortzakis and Economakis [15] also observed a significant spore germination inhibition of *Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarium* and *Rhizopus stolonifer*. However the bio pesticide failed to inhibit spore germination of *Aspergillus niger* [15]. These results disagreed with Yousef [16] who observed that the bio pesticides have some fungicidal activity against the *Aspergillus spps*.

Studies have been conducted to determine the active ingredients in *C. citratus* and was found to comprise alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids [17] and these phytochemicals are well-known for numerous protective and healing properties [18]. The citral which is a major component in the lemongrass is known to increase chitinase activity and thereby leading to cell walls degradation, therefore the mode of action for citral may be its capacity to cause significant harm of fungal pathogens [19]. The results were also in line with Srivastava et al. [20] who pointed out that *C. citratus* have both anti-fungal and anti-bacterial properties.

Aloe vera (*A. barbadensis)* has also shown potential in controlling Coffee Leaf Rust starting from the lowest concentration (25%). The botanical at its neat (100%) concentration was not significantly different from Copper oxychloride. Comparing the performance of the botanicals in inhibiting the spores from germination, Aloe vera was the least though not significantly different from tobacco at their 100% extract and Copper oxychloride. The *A. barbadensis* is an ancient pharmaceutical plant recognised for its anti-inflammatory, wound healing, anti-fungal, anti-bacterial anti-cancer properties and also immune boosting [21]. According to Parvu and Parvu [22],

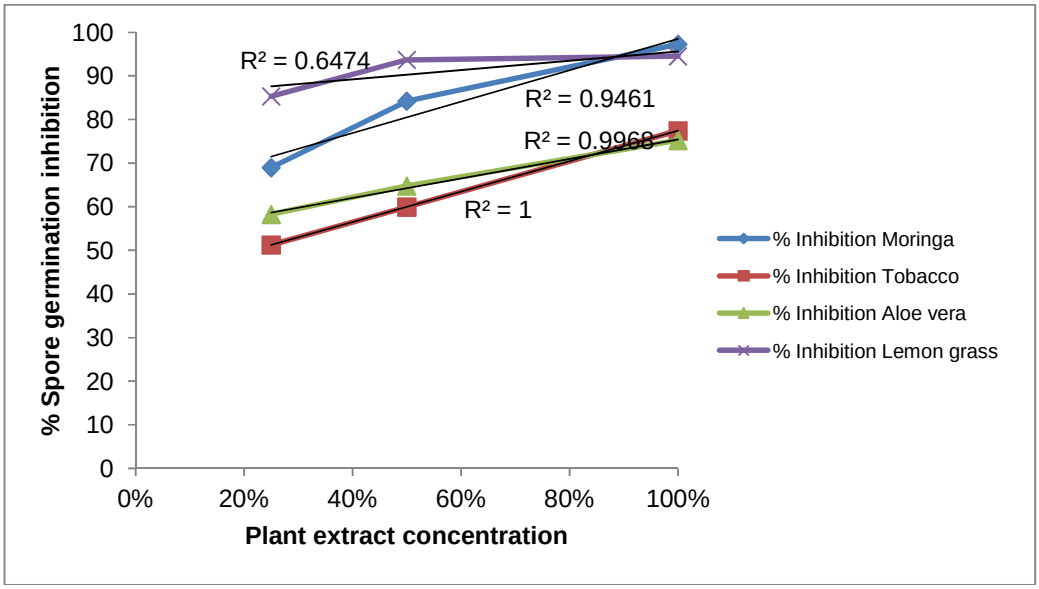

Fig. 1. Correlations between plant extract concentrations and % spore germination inhibition

A. barbadensis extract was found to have inhibitory effects against the growth of *Fusarium oxysporum* in artificial media. Aloe vera comprises six antiseptic agents viz lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur, all of the phytochemicals have inhibitory effect on fungi, bacteria and viruses [22]. According to Martinez [23], the mode of action for phenolic compounds which are a component of Aloe vera disrupt cytoplasm membrane thus leading to the leakage of cells. This is in line with the findings by Onyeani et al. [24]; Nabigol and Asghari [25] who observed that *A. barbadensis* can significantly control storage fungi such as *Aspergillus fluvus*, *Aspergillus niger, Penicillium expansum and Rhizopus stolonifer*. The results of this experiment is also in agreement with a study by Rodriguez et al. [26] who observed that the Aloe vera pulp and liquid fraction have some fungicidal properties against *Rhizoctonia solani, Fusarium oxysporum* and *Choletotrichum coccodes* with the liquid fraction showing a broader range of antifungal action than the pulp. Earlier work by other researchers reported that Aloin and aloe – emodin are the main active ingredients which inhibit growth of fungi and other microbes [22].

Moringa oleifera (M. oleifera) 100% and 50% concentrations with 97.30 and 84.24% respectively) performed better than the standard fungicide (Copper Oxychloride 85% WP) with 72.05% spore germination inhibition. The higher inhibitory effects exhibited by *M. oleifera* performance at its higher concentrations suggests that the botanical has a great potential in giving a better control to Coffee Leaf Rust than the standard fungicide. *M. oleifera* leaves and roots were evaluated and found to have fungicidal activity against *Fusarium oxysporum, Fusarium solani, Alternaria solani, Alternaria alternata, Rhizoctonia solani, Sclerotium rolfsii and Macrophomina phaseolina* [27]. The performance of *M. oleifera* was found to be in line with other studies done on potentiality of *M. oleifera* extracts from leaves, seed, flowers and stem bark in the control of coffee wilt pathogen (*Gibberella xylarioides*) [28]. The results of the study revealed that the extracts from all the Moringa parts exhibited broad fungicidal activity. The pesticidal performance of the Moringa leaf extract can be attributed to the presence of the phytochemical compounds such as tannins, flavonoids, steroids, terpenes and alkaloids which is rich in *Moringa oleifera* leaves and these dietary flavonoids possess antiviral, anti-inflammatory, antihistamine and antioxidant properties [29,30]. Nwekwe (2015) reported that tannins present in *Moringa oleifera* inhibited cell formation in fungi leading to the death of microbes. Inhibitory effect of Moringa leaf extracts increase with increase in concentrations [31]. Nwangburuka et al. [32] did a related work when testing *Moringa oleifera* leaf extract and sodium hypochlorite seed pre-treatment on the germination of seed, growth rate of seedling and the abundance of fungi in two accessions of *Abelmoschus esculentus* (L) Moench. The study

revealed that seed treatment before storage with Moringa decreases the probability of fungal infection and also sustains the viability and vigour of the seed for a certain time period, depending on the seed type.

Nicotiana tabacum (*N. tabacum*) also managed to control Coffee Leaf Rust spore germination *in vitro*. Copper oxychloride 85%WP and undiluted tobacco extract did not differ significantly from each other suggesting that tobacco has potential in controlling Coffee Leaf Rust control. Only 100% tobacco extract was comparable to Copper Oxychloride with all other concentrations weaker when compared to the standard fungicide. The inhibitory effect may be due to the presence of the alkaloid (nicotine) compound which is both insecticidal and anti-fungal [33,34]. The results indicated that increasing the active ingredient results in the increase in spore germination inhibition. The inhibitory effects of tobacco leaf extracts were in line with other studies done on the efficacy of Tobacco, Neem, Mexican marigold and Peri winkle extracts in controlling *Fusarium* yellows in common beans. The results of that study indicated that tobacco extract failed to control the pathogenic fungi as it did not differ significantly from the untreated control (sterile distilled water) [33]. However, in a related experiment tobacco extract was found to inhibit *Aspergillus viridae, Penicillium digitatum* and *Rhizopus* spp when tested alongside with Neem extract [35].

5. CONCLUSION AND RECOMMENDATIONS

From the research it can be concluded that the different plant extracts used in this study are effective in significantly inhibiting fungal rust spore germination percent. As the concentration of the plant extracts increased, its effect on spore germination inhibition percent also increased. Of the four botanical extracts used, Moringa was found to have the highest inhibitory effect though this was not significantly different from Lemon grass at 100%. Aloe vera had the lowest spore germination inhibitory effect. It is recommended that high concentrations of plant extracts (50-100%) be used in order to ensure effective fungal spore inhibition. Use of plant extracts as a method of controlling *Hemileia vastatrix* can be of great advantage. It is recommended that further research be done on the different plant extracts so as to identify the active compounds which are present in the extracts as these are responsible for the fungicidal effect. Furthermore, it is recommended that more studies be done to test the antifungal activity of the studied extracts on other different fungi using different concentrations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Arneson PA. Coffee rust. The Plant Health Instructor; 2000.
   DOI: 10.1094/PHI-I-2000-0718-02
2. Brown J, Whan J, Kenny M, Merriman P. The effect of coffee leaf rust on foliation and yield of coffee in Papua New Guinea. Crop Protection. Elservier Science Ltd. 1995;14(7): 589-592.
3. Rutherford MA, Phiri N. Pests and diseases of coffee in Eastern Africa: A technical and advisory manual. CAB International, Wallingford; 2006.
4. Daivasikamani S. Comparative efficacy of systemic fungicides for control of coffee leaf rust in India. Proceedings of the International Scientific Symposium on Coffee, December 4, Bangalore, India; 2000.
5. Steiman SA. *Hemileia vastatrix*; 2006.
   Available:http://www.coffeeresearch.org/agriculture/coffeeplant.htm
   (Accessed on 15 December 2015)
6. Hindorf H, Omondi CO. A review of three major fungal diseases of *Coffea arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya. Journal of Advanced Research, Ruiru, Elservier B.V. 2011;109.
7. Zambolin L, Zambolim E, Varzea V. Durable resistance to coffee leaf rust. Universidade federal de Vicosa, Portugal; 2005.
8. Chidoko C. An evaluation of agriculture and economic performance in Zimbabwe. Research Journali's Journal of Commerce, 2013;1(2):5.
9. Subramani D, Rajanaika J, Chinnaswamy KK, Singh S, Kumar VKP, Bhat SS, Jayarama. Comparative efficacy of plant products on the spore germination and disease incidence of coffee leaf rust pathogen, Karnataka, Central Coffee Research Institute; 2012.
10. Rainforest Alliance. List of Prohibited Pesticides, San Jose, Sustainable Agriculture Network Secretariat; 2009.

11. Babu J, Muzafar AD, Kumar V. Bioefficacy of plant extracts to control Fusarium solani melongenae of Brinjal wilt. Global Journal of Biotechnology and Biochemistry. 2008; 3:56-59.
12. Gurjar MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management. Journal of Agricultural Sciences. 2012;3(3):426.
13. Singh PK, Kumor V. Effectiveness of plant extracts in controlling wilt pathogen of chrysanthemum. Bioscience Discovery. 2011;02(2):232-235.
14. Nambiar VS, Matela H. Potential functions of lemon grass (*Cymbopogon citratus*) in health and disease. International Journal of Pharmaceutical and Biological Archives. 2012;3(5):1035-1043.
15. Tzortzakis NG, Economakis CD. Antifungal activity of lemon grass (Cympopogon citratus) essential oils against key postharvest pathogens, Chania, Department of Hydroponics and Aromatic plants, Institute of Olive Tree and Subtropical Plants, National Agricultural Research Foundation; 2007.
16. Yousef SAA. Antifungal activity of volatiles from lemon grass (*Cympopogon citratus*) and Peppermint (*Mentha piperta*) Oils against some respiratory pathogenic species of Aspergillus. International Journal of Current Microbiology and Applied Sciences. 2013;2(6).
17. Shah G, Shri R, Panchal V, Sharma N, Singh B. Scientific basis for the therapeutic use of *Cymbopogon citratus*. Journal Advanced Pharmacertical Technolology Research. 2011;2(1):3–8.
18. Asaolu MF, Oyeyemi OA, Olanlokun JO. Chemical compositions, phytochemical constituents and *in vitro* biological activity of various extracts of cymbopogon citrates. Pakistan Journal of Nutrition. 2009;8(12): 1920-1922.
19. Li RY, Wu XM, Yin XH, Liang JN, Li M. The natural citral can cause significant damage to the hyphal cell walls of magnaporthe grisea. Moleclues. 2014; 19(7):10279-90.
    DOI: 10.339/molecules190710279
20. Srivastava V, Dubey S, Mishra A. A revew on lemon grass: Agricultural medicinal aspect. International Journal of Pharmacy. 2013;4(8).
21. Lakshmi PTV, Rajalakshmi P. Identification of phyto-components and its biological activities of aloe vera through the gas chromatography-mass spectrometry. International Research Journal of Pharmacy. 2011;2(5):247-249.
22. Parvu M, Parvu AE. Antifungal plant effects, science against microbial pathogents. Communicating Current Research and Technological Advances. 2011;1055-1062.
23. Martinez JA. Natural fungicides obtained from plants. Fungicides for Plant and Animal Diseases. Dhanasekaran D. (ed); 2012.
    Available:http://www.intenchopen.com/books/fungicides-for-plants-and -animals-from-plants
    Accessed on 21 May 2015.
    ISBN: 978-953-307-804-5
24. Onyeani CA, Osunlaja SO, Owuru, Joda AO. Evaluation of Aqueous plant extracts in the control of storage fungi. International of Scientific and Technology Research. 2012;1(6).
25. Nabigo IA, Asghari A. Antifungal activity of aloe vera gel on quality of minimally processed pomegranate arils. International Journal of Agronomy and Plant Production. 2013;4(4).
26. Rodriguez D, Hernandez CD, Rodriguez GR, Angulo SJI. Antifungal activity *in vitro* of aloe vera pulp and liquid fraction against plant pathogenic fungi. Industrial Crops Production. 2005;21(21):81-87.
27. Mohamedy RE, Abdalla AM. Evaluation of antifungal activity of *Moringa oleifera* extracts as natural fungicide against some plant pathogenic fungi *In vitro.* Journal of Agricultural Technology. 2014;10(4):963-982.
28. Nkya JW, Erasto P, Kilambo D, Chacha M. *In vitro* evaluation of antifungal activity of *Moringa oleifera* Lam extracts against coffee wilt pathogen, *Gibberella xylarioides*, Heim and Saccas. American Journal of Research Communication. 2014;2(12).
29. Nwekwe FE. Antifungal activity of petroleum extracts of *Moringa oleifera* leaves and stem bark against some plant pathogenic fungi. Journal of Natural Science Resource. 2015;5(8).
    Available:www.iiste.org
30. Madukwe EU, Ezeugwu JO, Eme PE. Nutrient composition and sensory evaluation of dry *Moringa oleifera* aqueous extract. International Journal of Basic and Applied Sciences. 2013;13(3).

31. Ojo JA, Olunloyo AA, Akanni EO. Efficacy of *Moringa oleifera* leaf powder against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) on stored cowpea (*Vigna unguiculata* L. Walp). Researcher. 2013;5(12):240-244.
32. Nwangburuka CC, Oyekale K, Ezekiel CN, Anokwuru PC, Badaru O. Effects of *Moringa oleifera* leaf extract and sodium hypochlorite seed pretreatment on seed germination, seedling growth rate and fungal abundance in two accessions of *Abelmoschus esculentus* (L) Moench. Archives of Applied Science Research. 2012;4(2).
33. Obongoya BM, Wagai SO, Odhiambo G. Fungitotic properties of four crude plant extracts *Fusarium oxysporum*. African Journal of Food Agriculture Nutrition and Development. 2009;9(8): 1652-1666.
34. Sarmamy A, Hashim H, Sulayman A. Insecticidal effects of some aqueous plant extracts on the control of Khapra *Trogoderma granarium* Evert. International Conference on Chemical, Biological and Environment Sciences (ICCEBS'2011) Bangkok. 2011;288-292.
35. Puripattanavong J, Songkram C, Lomlim L, Amnuaikit T. Development of concentrated emulsion containing *Nicotiana tabacum* extract for use as pesticide. Journal of Applied Pharmaceutical Science. 2013; 3(11):016-021.

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