Journal of Agriculture and Ecology Research International
11(2): 1-8, 2017; Article no.JAERI.28371
ISSN: 2394-1073

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Antifungal Effects of Botanical Leaf Extracts of *Lantana camara*, *Moringa oleifera*, and *Tagetes minuta* on *Rhizopus stolonifer in vitro*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2017/28371
Editor(s):
(1) Mirza Hasanuzzaman, Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Bangladesh.
Reviewers:
(1) Charu Gupta, Amity University, UP, India.
(2) Omar Mohammad Atrooz, Mutah University, Jordan.
Complete Peer review History: http://www.sciencedomain.org/review-history/18150

Original Research Article

Received 16th July 2016
Accepted 25th August 2016
Published 10th March 2017

ABSTRACT

Aims: This research aims at testing the efficacy of different botanical leaf extracts, (*Lantana camara*, *Moringa oleifera* and *Tagetes minuta)* on soft rot fungi, *Rhizopus stolonifer in vitro*.
Study Design: The experiment was carried out in a 3*2 Factorial arrangement +2 controls in a Complete Randomized Design replicated three times.
Place and Duration of Study: Department of Agronomy Laboratory, Midlands State University, Gweru, Zimbabwe. The research was done between April 2016 and May 2016.
Methodology: Antifungal activity of the plant extracts was tested using the poisoned food technique. Colony diameter, percentage inhibition of the growth of mycelium and identification of functional groups in botanical leaf extracts were done. Mycelia growth diameter of the *R. stolonifer* was measured at day 3, 5, and 7 after inoculation. Identification of functional groups was done using the FT-IR Spectroscopy.
Results: There was an interaction between botanical plant type and concentration rate. *L. camara* at 60% concentration was most effective in controlling *R. stolonifer*, with a colony diameter of (4.3

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cm) and an inhibition percentage of (48%). *M. oleifera* and *T. minuta* at 30% concentration gave the lowest colony growth inhibition of 30% and 31% respectively. FT-IR Spectroscopy confirmed the presence of phenols, alkanes, alkenes, anhydride and alkyl halide in all the three extracts. Amines were only detected in *L. camara* extract.

Conclusion: The data obtained provide additional information in support of plant extracts for control of *R. stolonifer,* though the efficacy of plant extracts tested still remain below that of the synthetic pesticide, (control). As the concentration of the extracts increased, the effectiveness of the extracts also increased.

Keywords: Botanical leaf extracts; Rhizopus stolonifer; FT-IR Spectroscopy.

1. INTRODUCTION

Postharvest losses as a result of fungal infection occur when products are stored for an extended period of time at cold or high temperatures or as a result of mechanical damage during storage or transport [1]. Post-harvest diseases are posing a major problem to the agriculture industry, where they account for about 50% losses in fruits stored in poor storage conditions, [2]. The most important fungi causing post-harvest diseases of plants are *Aspergillus* spp., *Alternaria* spp. and *Rhizopus stolonifer* [3]. *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. is the causal agent of *Rhizopus* rot disease in various fruits and vegetables, [4]. The fungi sometimes cause catastrophic rots as sporangiospores in air, water and land are disposed on wounds of fruits and vegetables after postharvest, [5]. *R. stolonifer* secretes pectinolytic enzymes which break down and dissolve pectic substances of middle lamella that hold the plant cells in place in the tissues [6]. This results in loss of vegetable and fruit quantity and quality. *Rhizopus* soft rot diseases destroy on average 10-30% of the total yield of crop, with more than 30% being recorded in developing countries, [7].

Reduction of post-harvest food losses is a critical component of ensuring future global food security, [8]. Post harvest handling methods have been used to prevent and control the *Rhizopus* soft rot which involves the use of fungicides, manipulation of the postharvest environment, hygiene practices, prevention of injuries and heat treatments, [9]. Synthetic fungicides have been mainly used for the control of *Rhizopus* soft rot. Despite their popularity and extensive use, serious concerns about health risks arising from the exposure of farmers when mixing and applying pesticides or working in treated fields and from residues on food and in drinking water for the general population have been raised, [10,11]. Most of the effective fungicides also are expensive and unaffordable to resource constrained smallholder farmers [12]. Research has been done using different botanical extracts on fungal diseases. Natural products present many advantages in terms of sustainability, mode of action and toxicity compared to chemical pesticides, [13]. The objective of this study was to evaluate the potential of *Tagetes minuta*, *Moringa oleifera* and *Lantana camara* on reduction of *R. stolonifer* growth *in vitro*.

2. MATERIALS AND METHODS

2.1 Research Site

The experiment was carried out under laboratory conditions at Midlands State University in Gweru, Zimbabwe.

2.2 Experimental Design

The experiment was laid out in a 3*2 Factorial arrangement + 2 controls in a Complete Randomized Design replicated three times. Mancozeb was used as the positive control and distilled water as the negative control. The experiment had two factors; botanical type (*L. camara*, *M. oleifera* and *T. minuta*) and extract concentration (30% and 60%).

Table 1. Treatments

Botanical type	Concentration %
M. oleifera	30
M. oleifera	60
L. camara	30
L. camara	60
T. minuta	30
T. minuta	60
Mancozeb (Control)	
Distilled water	

2.3 Source of *R. stolonifer* Fungi Inoculum

Rotting tomato fruits were obtained from the Green house of Midlands State University and

kept in a laboratory and where used to serve as source of fungal inoculum.

2.4 Isolation and Identification of Fungi from Rotten Tomato Fruits

Agar plate method developed by Narayanasamy 2011 [14] with slight modification was used to isolate the fungal organisms from the tomato fruits. Small segments of tissues (2 mm²) from the margin of rotted areas were cut using sterile scalpel and transferred into a prepared Potato Dextrose Agar (PDA) plates. The plates were incubated at 25±2°C for 5-7 days and the isolated fungal colonies were purified by sub-culturing.

Morphological and microscopic characteristics of the pure cultures were used for identification of the isolates following standard references [4]. The colony morphology used includes color of spores, presence or absence of pigmentation, elevation and nature of mycelia. Microscopic characteristics used for the identification include the type and shape of asexual and sexual spores, presence or absence of cross walls in hyphae, presence or absence of sterigmata and the sporangiophores.

2.5 Preparation of Extracts from *L. camara*, *M. oleifera* and *T. minuta* Leaves

Fresh leaves of *L. camara*, *M. oleifera* and *T. minuta* were harvested locally within the Midlands State University campus, rinsed in distilled water and air dried for three days. The leaves collected were at flowering stage of growth and one kilogram was weighed. Two hundred and fifty grams of air dried leaves were mixed with 250 ml of distilled water in a two litre volumetric flask and the mixture was homogenized for 10 minutes in a warring blender and sieved with sterile muslin cloth folded three times. The liquid obtained in each case was centrifuged at 5000 rpm for 30 minutes, after which the supernatants were decanted off and the sediments were collected for use, [15]. Ten grams of sediments were mixed with 10 mls of distilled water and shaken vigorously to produce 100% concentration. 30% and 60% concentrations were then prepared.

2.6 Inoculation of Fungal Isolates in Plates with PDA and Leaf Extracts

Two perpendicular lines were drawn at the bottom of the Petri dishes. The point of intersection indicates the centre of the plates. This was done before dispensing the PDA into each of the plates. The botanical leaf extracts of *L. camara, M. oleifera* and *T. minuta* were poured into the flask, plugged with cotton and heat sterilized to avoid contamination. Botanical plant extracts at a volume of two ml were introduced into the Petri dishes containing the media. The pure isolate of *R. stolonifer* was placed at the point of intersection of the two perpendicular lines drawn at the bottom of the plate.

2.7 Identification of the Functional Groups from the Botanical Leaf Extracts

Identification of functional groups was done using Fourier Transform Infrared Spectroscopy (FT-IR). Botanical leaves of *L. camara, M. oleifera* and *T. minuta* were collected from Midlands State University Campus. Fresh leaves were weighed and 1kg of each plant leaf type where rinsed with distilled water and air dried for three days. The dried leaves were blended into very fine powder and weighed (0.01g). Pure Potassium Bromide (KBr) was crushed using an agate pestle and motor and 0.1g was weighed. The leaf extract sample was mixed with KBr at a ratio of 1 (powdered sample):100 (KBr). The sample mixture was then grinded together for about three minutes after which it was compressed in a barrel tightened at both ends to make pellets or discs. The pellets were then one at a time put into the FT-IR sample holder and absorption and percentage transmission were recorded, [16].

2.8 Data Collection

2.8.1 Mycelia growth diameter

A 30 cm rule was used to measure the diameter starting at the point of intersection marked by the two perpendicular lines on Petri dishes as previously mentioned. Measurements were recorded at day 3, 5 and 7.

2.8.2 Inhibition of growth percentage

Inhibition growth percentage of the botanical leaf extracts to mycelia growth was calculated using the formula below [17];

$$\textit{Inhibition percentage} = (G_c - G_t / G_c)*100$$

Where $G_{c\ =}$ mean mycelia diameter in control plate and $G_{t=}$ mean mycelia diameter in extract plates

2.8.3 Identification of functional groups in the botanical extracts

The FT-IR spectra for all the three botanical leaf powders extracts were obtained and the effective peaks were compared with the IR frequency absorptions from literature. The peak value in the region of infrared radiation was used to identify the functional groups of the active components. The specific wave numbers lengths and intensities were considered.

2.9 Statistical Analysis

Data on mycelia diameter and inhibition growth percentage was analysed using Genstat version 14. Significantly different treatment means were separated using LSD at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Effects of Botanical Leaf Extracts (*L. camara, M. oleifera* and *T. minuta*) on *R. stolonifer* Colony Diameter

There was an interaction (P = 0.002) between botanical leaf type and concentration level on colony diameter. *L. camara* resulted in the lowest colony diameter at 60% concentration of the extract. *T. minuta* at 60% concentration gave the lowest colony diameter. There were no statistical differences among all the *M. oleifera* and *T. minuta* treatments on their effects on the growth of *R. stolonifer* mycelium.

3.2 Effects of Botanical Leaf Extract (*L. camara*, *M. oleifera* and *T. minuta*) on Growth Inhibition Percentage of *R. stolonifer*

There was an interaction (P = 0.006) between botanical leaf extracts and concentration on inhibition of growth percentage of *R. stolonifer*. There was a significant difference (P = 0.006) on the effects of different botanical leaf extracts on inhibition growth percentage of *R. stolonifer*. *M. oleifera* and *T. minuta* extracts at 30% concentration had the least inhibition of growth percentages, (30% and 31% respectively). *L. camara* at 60% concentration gave the highest inhibition of growth percentage of 48%, (Fig 2). Generally, growth inhibition percentage increases with an increase in concentration.

3.3 Identification of Functional Groups

3.3.1 Identification of functional groups in *M. oleifera*

The identification of functional groups was based on the FT-IR peaks attributed to stretching and bending vibrations. The FT-IR Spectra for *M. oleifera* leaf extract powder had characteristic absorption bands of 3439.14 cm^{-1}, 1635.45 cm^{-1}, 1384.93 cm^{-1}, 1075.32 cm^{-1} and 605.21 cm^{-1}, (Fig. 3). The peak at 3439.14 cm^{-1} confirmed a strong intensity of alcohols and phenols. The peak at 1635.45 shows the presence of alkenes, though the intensity is variable. Alkanes were

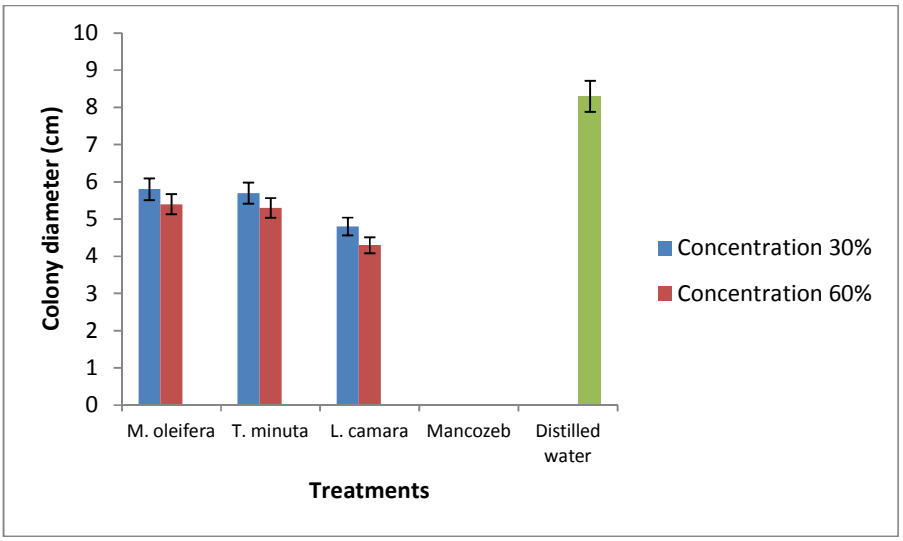

Fig. 1. Effects of botanical leaf extract (*L. camara*, *M. oleifera* and *T. minuta*) and concentration on colony diameter

also presence (1384.93 cm^{-1}) and the intensity at this range is also variable. The peak at 1075.32 cm^{-1} confirmed a strong intensity of anhydride. A strong intensity of alkyl halide was also noted at a peak of 605.21 cm^{-1}.

3.3.2 Identification of functional groups in *L. camara*

L. camara characteristic absorption bands of 3413.61 cm^{-1}, 2928.09 cm^{-1}, 1639.44 cm^{-1}, 1384.46 cm^{-1}, 1258.53 cm^{-1}, 1071.15 cm^{-1} and 619.88 cm^{-1}, (Fig 4.). A strong intensity of alcohols and phenols was detected (3413.61 cm^{-1}). There was also a strong intensity of alkanes, (2928.09 cm^{-1}). The peak at 1639.44 cm^{-1} showed the presence of alkenes though the intensity was variable. Amines (1258.53 cm^{-1}) were detected though the intensity was medium to weak. Strong intensity of anhydride and alkyl halide was also confirmed (1071.15 cm^{-1} and 619.88 cm^{-1}) respectively.

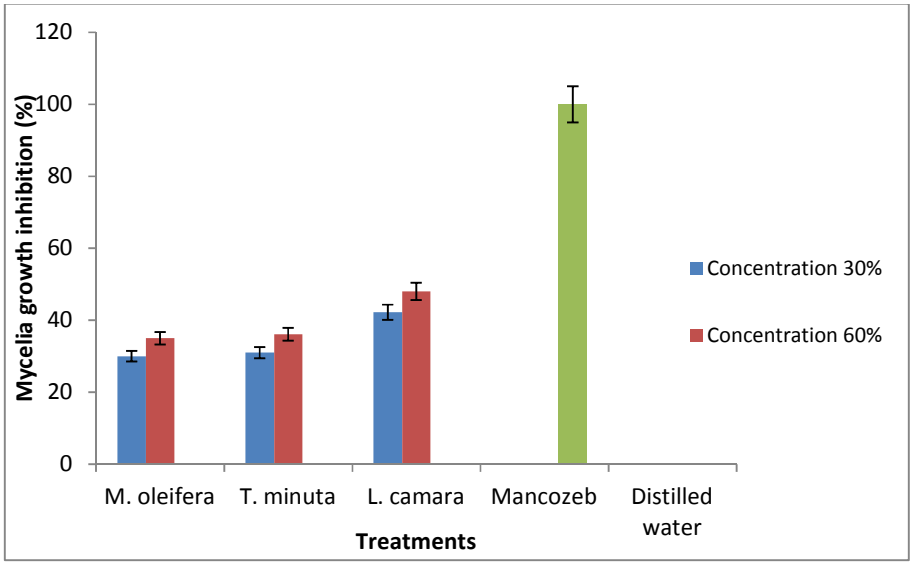

Fig. 2. Effect of botanical leaf extracts (*L. camara*, *M. oleifera* and *T. minuta*) on inhibition growth percentage of *R. stolonifer*

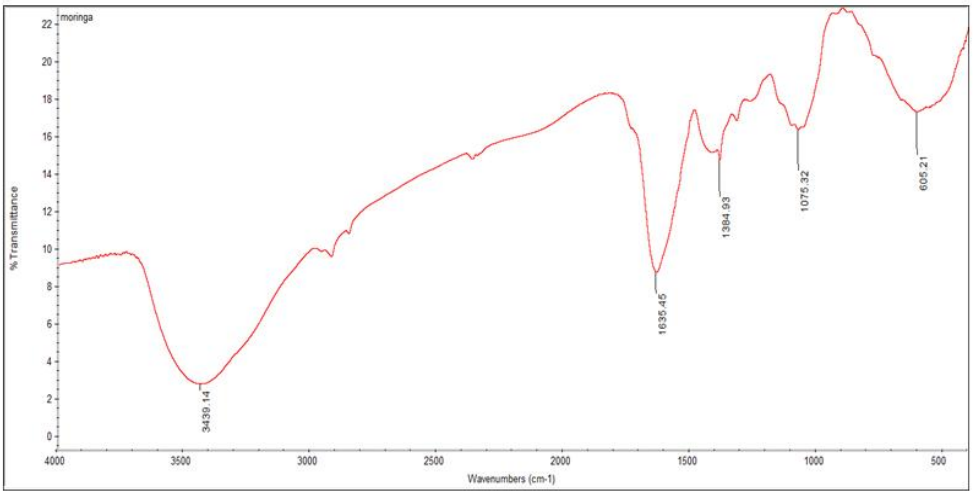

Fig. 3. FT-IR spectra of *M. oleifera* leaf extract with wave numbers ranging from 500 cm^{-1} to 4000 cm^{-1}

3.3.3 Identification of functional groups in *T. minuta*

Tagetes minuta had characteristic absorption bands of 3415.87 cm^{-1}, 29625.05 cm^{-1}, 1637.91 cm^{-1}, 1384.56 cm^{-1}, 1074.15 cm^{-1} and 619.59 cm^{-1}. The peak at 3439.14 cm^{-1} confirmed a strong intensity of alcohols and phenols. The peak at 1637.91 cm^{-1} shows the presence of alkenes, though the intensity was variable. A strong intensity of Alkanes (29625.05 cm^{-1}) was also confirmed. The peak at 1042.58 cm^{-1} confirmed a strong intensity of anhydride. A strong intensity of alkyl halide was also noted at a peak of 619.59 cm^{-1}.

3.4 Discussion

From the investigations, a reduction in *R. stolonifer* mycelia growth was observed due to the effect of botanical plant extracts. According to Naz and Bano [18] these effects are attributed to allelochemical activity. The studied plants contained bioactive compounds which include alcohols, phenols (phenolics), alkenes, alkanes, anhydrides and alkyl halide. However, the specific compounds in these broad groups could not be determined. Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently

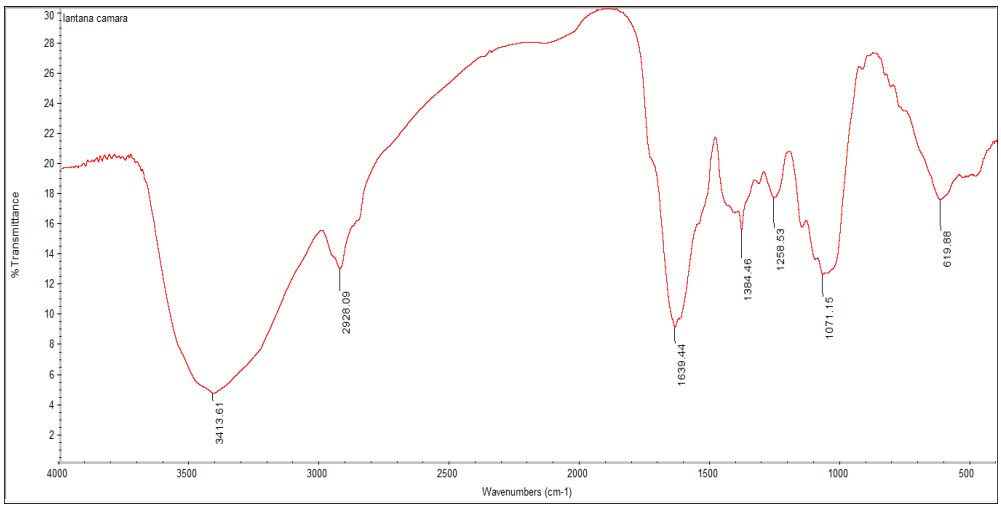

Fig. 4. FT-IR spectra of *L. camara* leaf extract with wave numbers ranging from 500cm^{-1} to 4000 cm^{-1}

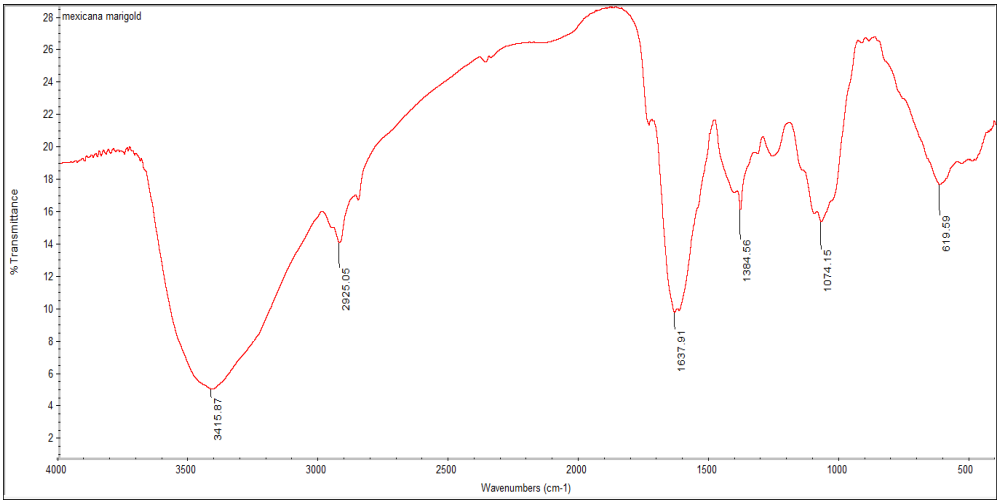

Fig. 5. FT-IR spectra of *T. minuta* leaf extract with wave numbers ranging from 500cm^{-1} to 4000 cm^{-1}

known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against pathogens [19]. The effect of phenols on *Rhizopus* spp is direct interaction with the ergosterol, the main sterol present in the fungi and is essential for the proper growth and development of it. This leads to the disruption of the membrane integrity, fluidity and loss of the intracellular content that leads to the mortality of the fungal pathogen [20]. Tsukamoto et al. 1994 [21] reported the antifungal activity of alkenes and alkanes.

L. camara at 30% concentration gave the lowest colony diameter as well as the highest inhibition of mycelia growth of 48%. In addition to the phenols, alkanes and alkenes, *L. camara* also contained amines. Subík et al. [22] documented the antimicrobial activity of amine oxides were inhibition of growth of different forms of filamentous fungi was observed. However, the FT-IR spectra does not give the specific types of amines present so that the actual effect on *R. stolonifer* can be explored. For all the plant extracts tested, the higher concentration (60%) gave a higher inhibition of the growth of mycelium. This could be due to the higher concentration of the biologically active compounds in the plant extracts. Though *L. camara* at 60% concentration gave the highest inhibition percentage (48%), this was 52% lower than the standard chemical pesticide (Mancozeb). In a research by Al-Rahmah et al. [23] it was noted that, although *L. camara* was effective in controlling *Pythium aphanidermatum* at 10 mg ml-1, it was ineffective in controlling the other tested fungal species and higher concentrations more than 10 mg ml-1 were required to be effective. This suggests that *L. camara* could be more effective at a concentration higher than 60%, which was this highest concentration used in this research.

The low efficacy of the extracts might also be due to the extraction methods used. In a study by Boeing et al. [24] it was observed that acetone/water (70/30, v/v) solvent mixture was more efficient solvent in the extracting of phenolic compounds than other solvents. This shows that the solvent used for extraction of plant active compounds has an effect on the type and amount of biologically active compounds obtained.

4. CONCLUSION

The results showed that the least diameter for mycelia growth was 4.3 cm, (*L. camara* at 60% concentration). *M. oleifera* and *T. minuta* at 30% concentration gave the lowest colony growth inhibition of 30% and 31% respectively. FT-IR Spectroscopy confirmed the presence of phenols, alkanes, alkenes anhydride and alkyl halide in all the three extracts, amines were only detected in *L. camara* extract. The method, (FT-IR Spectroscopy), however, does not quantify the amounts of active compounds in the plant extracts. In future research, it is therefore recommended to quantify the active compounds in the plant extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agrios GN. Plant pathology. 5th ed. New York: Academic Press; 2005.
2. Maswada HF, Abdallah SA. *In vitro* antifungal activity of three geophytic plant extracts against three post-harvest pathogenic fungi. Pakistan Journal of Biological Science. 2013;16:1698-1705.
3. Ogawa JM, Mathre JH, Weber DJ, Lyda SD. Effects of 2,6-dichloro-4-nitroaniline on *Rhizopus* species and its comparison with other fungicides on control of *rhizopus* rot of peaches. Phytopathology. 1963;(53) 950-955.
4. Hernández-Lauzardo AN, Velázquez-Del Valle MG, Veranza-Castelán L, Melo Giorgana GE, Guerra-Sánchez MG. Effect of chitosan on three isolates of *Rhizopus stolonifer* obtained of peach, papaya and tomato, Fruits. 2010;245–253.
5. Agrios George N. Plant pathology, 4th edition. Academic Press. San Diego, CA; 1997.
6. Salman MAM. Biological control of *Rhizopus* soft rot on apple, pear and peach by *Trichoderma harzianum.* An-Najah National University Faculty of Graduate Studies; 2005.
   (Accessed 01/07/2016)
   Available:https://scholar.najah.edu/sites/default/files/all-thesis
7. Hamim I. Non-chemical approaches of managing postharvest fungal diseases of ripe papaya; 2013.

(Accessed 01/07/2016)
Available:http://dspace.bau.edu.bd/bitstream/123456789/1455/1/P%20PATH-747%20JUNE- 2013.pdf
8. Trostle R. Global agricultural supply and demand: Factors contributing to the recent increase in food commodity prices. Rev. DIANE Publishing; 2010.
9. Kader AA, Rolle RS. The role of post-harvest management in assuring the quality and safety of horticultural produce. Food and Agriculture Organization of the United Nations Rome; 2004.
10. Maroni M, Fanetti AC, Metruccio F. Risk assessment and management of occupational exposure to pesticides in agriculture. Med. Lav. 2006;97:430–437.
11. Soares WL, Porto MFD. Estimating the social cost of pesticide use: An assessment from acute poisoning in Brazil. Ecol. Econ. 2009;68:2721–2728.
12. Buluswar S, Friedman Z, Mehta P, Mitra S, Sathre R. Critical scientific and technological advances needed for sustainable global development. Institute for Globally Transformative Technologies, Lawrence Berkeley National Lab.
(Accessed 13/07/2016)
Available:https://ligtt.org/sites/all/files/page/50BTsFoodSecurityAndAgriculturalDevelopment_0.pdf
13. Nega A. Review on concepts in biological control of plant pathogens. Journal of Biology, Agriculture and Healthcare. 2014; 4(27).
14. Narayanasamy P. Microbial plant pathogens-detection and disease diagnosis. Fungal Pathogens. 2011;90-481-9735-4.
15. Lalitha P, Sripathi SK, Jayanthi P. UV protecting ability of sunscreen lotions prepared with extracts of *Pisonia grandis* R. Br World Journal of Pharmacy and Pharmaceutical Sciences. 2015;4:324-329.
16. Ashokkumar R, Ramaswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. International Journal of Microbiology and Applied Sciences. 2014;2319-7706.
17. Singh J, Tripathi NN. Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. Flavour and Fragrance Journal. 1999;14:1-4.
18. Naz R, Bano A. Effects of allelochemical extracts from medicinal plants on physiological and biochemical mechanisms of maize (*Zea mays* L.) seedlings. International Journal of Agronomy and Agricultural Research (IJAAR). 2014:2-31-39.
19. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. 2010;15:7313-7352.
20. Mota KS, Pereira F, Oliveira WA, Lima IO, Lima E. Antifungal activity of *Thymus vulgaris* L. Essential oil and its constituent phytochemicals against *Rhizopus* oryzae: Interaction with Ergosterol. Molecules. 2012;17:14418-14433.
21. Tsukamoto S, Kato H, Hirota H, Fusetani N. Antibacterial and antifungal sulfated Alkane and alkenes from the hepatopancreas of the ascidian. Halocynthia roretzi. J. Nat. Prod. 1994; 57(11):1606–1609
22. Subík J, Takácsová G, Psenák M, Devínsky F. Antimicrobial activity of amine oxides: mode of action and structure-activity correlation. Antimicrob Agents Chemother. 1977;12(2):139-46.
23. Al-Rahmah AN, Mostafa AA, Abdel-Megeed A. Yakout SM, Hussein SA, Singh J, Tripathi NN. Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. Flavour and Fragrance Journal. 1999;14:1-4.
24. Boeing JS, Barizão EO, Silva BC, Montanher PF, Almeida VC, Visentainer JV. Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: Application of principal component analysis. Chemistry Central Journal. 2014; 8:48.

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