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Exploring Knowledge about Antifungal Property of Neem

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Abstract: *Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. This review summarises the wide range of pharmacological activities of neem leaf.[2].*

Keywords: Azadirachta indica.

I. INTRODUCTION

Fungi are the major cause of plant diseases and are responsible for large scale harvest failures in crops like maize and other cereals all over the world (Suleiman and Omafe, 2013). The fungi genera typically found in stored grains are Aspergillus, Penicillium, Fusarium and some xerophytic species, several of them with capabilities of producing toxins (Castellari et al., 2010). Seed fungi especially species of Aspergillus, Diplodia, Penicillium, Fusarium, Trichoderma and a number of phycomycetes affect the seed of all forest species. Previous studies by Pacin et al. (2009) identified Aspergillus and Fusarium as mycotoxigenic species in stored grains. So also, their mycotoxins and fumonisins were reported in different concentrations by Moreno et al. (2009) in stored grains. Chemical control of fungal pathogens has been of help in the increase of crop yield. However, usage of these chemical products is being discouraged due to the resultant environmental pollution which leaves toxic residues in soil, water and food. Some chemicals are also harmful to non-target organisms and this leads to ecological imbalance and development of fungicidal resistant strains. All these limitations call for an alternative plant disease management strategy such as biological control (Gardener and Fravel, 2002; Lokesha and Benagi, 2007).

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Biological control method is preferred because it is selective with no side effects, and is relatively cheap. Moreover, resistance to biological control is rare and biological control agents are self-propagating and self-perpetuating. Neem (Azadirachta indica) is a widely prevalent tree, mainly cultivated in India subcontinent (Karl, 1997). Various parts of the tree have been used as traditional Ayurvedic medicine in India (Brahmachari, 2004). Neem oil in particular was widely used as a traditional medicine in India, Sri Lanka, Burma, Thailand, Malaysia and Indonesia and already has more than 2000 years history. Neem oil was often administered orally, for deworming and constipation, and is applied topically to relieve rheumatism, ulcer, itching and cure chronic skin diseases (Aggarwal and Dhawan, 1995). There is evidence that neem oil has acaricidal, antibacterial, antifungal, antimalarial, antiparasitic, anti-inflammatory as well as immunomodulatory properties in different animal species (Mulla and Su, 1999; Biswas et al., 2002; Brahmachari, 2004; Gossé et al., 2005; Du et al., 2007, 2008, 2009; Xu et al., 2010; Zhang et al., 2010). Due to its efficacy, biodegradability and minimum side effects, azadirachtin, a tetranortriterpenoid obtained from neem seeds, has emerged as a natural biopesticide (Locke, 1995; Martinez, 2002). The objective of this study was to evaluate the efficacy of neem seed oil against selected pathogenic fungi species that constitute major threats to agricultural products in Nigeria.[4]

Chemical composition of Neem

Neem is a complex mix of various chemical constituents. Hossain et al. (42) have shown that the primary constituents of crude Neem leaf extract (NLE) include hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides [2-ethylhexyl tetradecyl est (13.70%), methyl petroselinate (11.23%), eicosane, 7-hexyl (10.01%), heptacosane (8.10%), hexadecamethylcyclo-octasiloxane (7.46%), octacosane (7.09%), heptacosane, 7-hexyl (6.77%), butyl palmitate (6.69%), isobutyl stearate (4.25%), nonadecane (3.75%), (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (2.99%), 2,6,10,14-tetramethylheptadecane (2.68%), phytol (2.61%), methyl isoheptadecanoate (2.19%), and gamma-elemene (1.05%)]. Multiple studies have shown that terpenoids (including limonoids) are primarily responsible for Neem's biological activities (Table 2). Chemical characterization of Neem extracts via LC-MS by Santos et al. (92) demonstrated the following terpenoids in Neem extracts; 2,3-Dihydronimbolide (22.8%), Nimbolide + 3-Deacetylsalannin (19.7%), Nimbandiol (12.8%), Nimonol (10.1%), 6-Deacetylnimbinene (9.3%), 6-Deacetylnimbin (8.4%), Gedunin (4.8%), Nembutal (4.4%), Salinan (3.9%), Ruthin (3.6%). It is noteworthy that four different extraction solvents were used in their study and while the percentage of each compound obtained in each extraction fraction was similar, their concentration varied depending on the polarity and capacity of different solvent used for the extraction.

Antifungal property of neem

Inhibitory e□ect on both standard isolated strains of S. aureus. In the case of E. faecalis, methanol and ethanol extracts revealed maximum growth inhibitory effects against the clinical and standard strains, respectively. Based on the MIC index effects, methanolic extract possessed bactericidal e□ect against both standard and nosocomial strains of P. aeroginosa and S. aureus and bacteriostatic e□ect against the nosocomial strain E. faecalis. The ethanolic extract exhibited bactericidal activity towards both standard and nosocomial strains of P. aeroginosa and E. faecalis and bacteriostatic activity against the nosocomial strain of S. aureus. Ethyl acetate extract had revealed bactericidal activity

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against standard strains of P. aeroginosa and S. aureus and bacteriostatic against the standard strain of E. faecalis and nosocomial strain of S. aureus. Thus, neem can be an e□ective therapeutic agent to 肆ght antibiotic-resistant bacteria (Maleki et al., 2018).Antifungal e□ect of neem extracts Alcoholic extract of neem leaves is bene肆cial in retarding Aspergillus and Rizopous fungal species (Srivastava et al., 2020). The antifungal properties of neem could also be found in oil and bark extracts and the other parts of neem extracts (Mahmoud et al., 2011). Another study was conducted to determine antifungal activity of various neem leaf concentrates on seed-borne parasites such as Aspergillus and Rhizopus; the 肆ndings showed that the growth of both infectious species was profoundly depressed and regulated by aqueous and alcoholic extracts (Giri et al., 2019). Moreover, an alcoholic neem leaf extract was tested.[5]Experiment was made to evaluate the efficacy of various extracts of neem leaf on seed borne fungi Aspergillus and Rhizopus and results confirmed that growth of both the fungal species was significantly inhibited and controlled with both alcoholic and water extract. Furthermore, alcoholic extract of neem leaf was most effective as compared to aqueous extract for retarding the growth of both fungal species . Another finding showed the antimicrobial role of aqueous extracts of neem cake in the inhibition of spore germination against three sporulating fungi such as C. lunata, H. pennisetti, and C. gloeosporioides f. sp. Mangiferae and results of the study revealed that methanol and ethanol extract of Azadirachta indica showed growth inhibition against Aspergillus flavus, Alternaria solani, and Cladosporium .Aqueous extracts of various parts of neem such as neem oil and its chief principles have antifungal activities and have been reported by earlier investigators . A study was undertaken to examine the antifungal activity of Azadirachta indica L. against Alternaria solani Sorauer and results confirmed that ethyl acetate fraction was found most effective in retarding fungal growth with MIC of 0.19 mg and this fraction was also effective than fungicide (metalaxyl + mancozeb) as the fungicide has MIC of 0.78 mg .[6]

II. ANTIFUNGAL PROPERTIES OF NEEM (AZARDIRACHTA INDICA) LEAVES EXTRACT TO TREAT HAIR DANDRUFF

Dandruff due to fungus is extremely common, affecti ng close to 50% of the world's population and it also most prevalent between ages 15 and 50. Thus, this study has been conducted to come up with Neem leaves extract that has high anti fungal properties. Neem is an attractive broad-leaved, evergreen tree which can grow up to 30m tall and 2.5m in girth. Its trunk usually straight is 30-80 cm in di ameter. Its spreading branches form a rounded crown of deep-green leaves and honey-scented flower s as much as 20m across. Neem is native of India, Pakistan, Thailand and Burma. Its actual origin is still debatable, but it is for sure that it originated in the Indian subcontinent and f rom there it spread to different parts of the world. The Neem leaves were macerated in 400 mL ethanol an d covered with parafilm. It was allowed to stand in room temperature for 48 hours then it w as filtered. To get 100% extract, an amount of the extract was placed in an evaporating dish to be subjected to water-bath. The extract looked dark brown in its liquid form. The fungi (P. ovale) was cultured in the Laboratory . Various levels of Neem extract concentration (25%, 50%, 75%, and 100%) were prepar d. The inhibiting capacity of each level on fungus that causes dandruff was tested usi ng agar cup method. To establish a firmer and more solid foundation of the contention that hi gh antifungal properties is present, experiment was conducted in three treatments. The 1 00% extract of Neem leaves produced the widest zone of inhibition which was found statistic ally highest than the other concentration levels.[9]

Materials

Neem seeds were collected in Kunming (Yunnan, China) during July of 2019. The seeds were washed and inside air-dried for one week. The wood fungi under investigation, Trametes versicolor and Gloeophyllum trabeum, were obtained from the Chinese Strain Preservation Center (Beijing, China). The fungi were preserved in potato dextrose agar (PDA) and stored at 4 ℃. The experimental tree (P. tomentosa) grew in the Yichun area in Heilongjiang province. Sapwood samples (20 mm × 20 mm × 10 mm) were dried to constant weight at 105 °C.

Method

Neem leaf extract was prepared by using absolute ethanol with fresh neem leaves, filtering the extract through muslin cloth, coarse residue and filter paper. Cultures of C. albicans were maintained on .Heart.Infusion broth and Agar. The

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antimicrobial efficacy was checked using the Agar Diffusion and the zones of inhibition were measured. The results were statistically analysed using ANOVA.

Preparation of Neem Seed Extract

The neem seeds were ground to 20-mesh. A total of 20 g of neem powder was mixed with 280 mL of 60% v/v ethanol solution (Chang et al. 2018). The mixture was stirred in a water bath at 50 ℃ for 90 min. The residual ethanol was evaporated using a vacuum rotary evaporator (RE52AA; Huanyu, Zhejiang, China). The extracts were stored at 4 ℃ for further use.

Agar Diffusion Assay

Petri dishes containing 15 mL of PDA were used for the antifungal activity assay, conducted on solid media using the disk diffusion method (Bajpai et al. 2008). The PDA solution was prepared using sterile distilled water, serially diluted, and added at final concentrations of 0.5%, 1%, 5%, 10%, and 15% to 5-mm diameter holes punched in the agar. The plates were incubated at 26 ℃ for 5 to 7 days. The inhibition zone were measured using a vernier caliper.[14]
test.Inter-group comparison was checked using Kruskal Wallis ANOVA and Mann Whitney tests (a=0.05).[1]

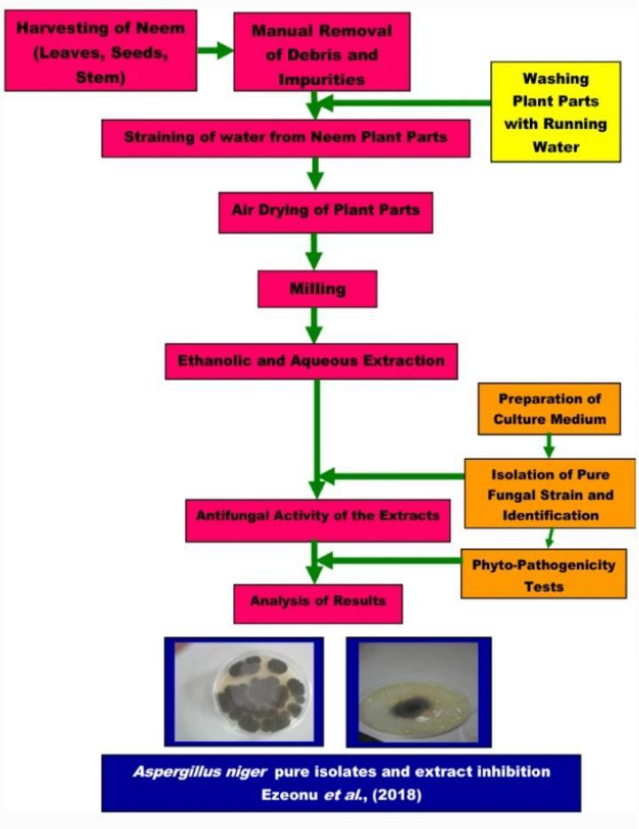

Fig:- Methods of preparation

III. RESULTS

From the yam, Aspergillus ochre's, Scleroderma rolfsii and Lasidiodiplodia theobromae were isolated, while Aspergillus niger, Aspergillus oryzae and Rhizopus stolonifer were isolated from cocoyam samples. After 14 days of inoculation, isolated fungi were shown to be highly pathogenic except R. stolonifer and A. ochareus, which showed moderate pathogenicity. All the plant extracts showed antifungal activities at varied percentage mean zones of inhibition. The inhibition against fungal growth from the isolates of the rotted cocoyam gave mean inhibition between 39.53 ± 8.39% and 96.76 ± 1.60%. Except for ethanolic stem bark extract which gave 39.53 ± 8.39% limited zone of inhibition against A. niger growth, the rest of the extract exhibited good zones of inhibition against cocoyam-isolated

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fungi ranging between 72.87 ± 3.55 and 96.79 ± 1.60%. On fungal isolates from yam, the mean percentage zones of inhibition of all extracts of the neem plant parts showed more effectiveness than the positive control (Ketoconazole) against L. theobromae growth. Aqueous stem bark extract with 93.80 ± 1.78% zone of inhibition showed comparable effectiveness to the control (100% inhibition) against A. ochareus growth, while against S. rolfii growth, there was good inhibition by all the extracts though not to the extent as that of the control.[9]

IV. CONCLUSION

The fungitoxic potential of these plant extracts on rot-inducing fungi of stored yam and cocoyam suggests that the above extracts used in this study can be put to use by farmers as alternative to commercial/synthetic fungicides.[9]

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