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RESEARCH ARTICLE

ANTIFUNGAL EFFECT OF *ZINGIBER ZERUMBET* EXTRACT AND ITS COMPATIBILITY WITH TOBACCO DECOCTION – A BIOPESTICIDE.

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Abstract

A study was conducted with the objectivity of assessing the antifungal property of *Zingiber zerumbet* and compatibility of extract of rhizome of this plant with tobacco decoction a widely used biopesticide by dual culture technique. Out of the six fungal strains tested, *Fusarium oxysporum*, *Corynespora cassiccola* and *Pithium* species were inhibited (64.72%, 50.26% and 50% respectively) by the methanolic extract. The methanolic extract of *Zingiber zerumbet* did not have any inhibitory effect against fungi viz. *Rhizopus*, *Colletotrichum gloeosporioides* and *Aspergillus niger*. Green house experiment was done with tomato saplings challenged with *Fusarium oxysporum*, a pathogen of tomato wilt. From the green house experiment done it was clear that the combination of *Zingiber zerumbet* extract with tobacco decoction acted as a better bio pesticide than tobacco decoction alone and its impact is markedly seen on different growth parameters of saplings analysed. The mixture can be supposed to be possessing fungicidal as well as insecticidal property.

Introduction:-

From time immemorial there have been number of medicinal, aromatic herbaceous and rhizomatous plants in use in different parts of the world in the field of ayuvedic medicine due to their potential pharmacological properties. Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhoea, cutaneous abscesses, bronchitis and parasitic diseases. Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable. Even though certain plants have been demonstrated for their effects on phytopathogenic fungi, a number of them have not yet been investigated for their antifungal activities. Hence, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs against the fungal pathogens, which are infecting economically important plants and trees, susceptible to these pathogens.

Zingiber zerumbet is a member of the family *Zingiberaceae* having certain antifungal activities. Traditionally tobacco decoction is used as an effective biopesticide against some of the pests and larvae found in plants. In view of the potential antifungal activity of *Zingiber zerumbet* and the increasing popularity of tobacco decoction as a biopesticide a study was carried out to assess the antifungal effects of *Zingiber zerumbet* against some phytopathogenic fungi and to test compatibility of the extract of *Zingiber zerumbet* with tobacco decoction by performing green house experiment with tomato plants.

Materials and Methods:-

Sample Collection:-

The rhizome *Zingiber zerumbet* were collected from the plain areas of Pulincunnoo in Alleppey district, Kerala. The samples were collected in the summer season in the month of April 2014. The phytopathogenic fungal strains like *Fusarium oxysporum*, *Pithium* species, *Aspergillus niger*, *Rhozoctonia zolani* and *Rhizopus* species were

obtained from the culture collection of School of Biosciences, M.G. University. Phytopathogenic fungi viz. *Colletotrichum gloeosporioides and Corynespora cassiccola* were produced from Rubber research institute, Kottayam, Kerala, India

Processing of Zingiber Zerumbet:-
30-40 gm of the tender rhizomes of *Zingiber zerumbet* was weighed. These rhizomes were thoroughly washed under running tap water. And they were rinsed in sterile distilled water. These rhizomes were surface sterilized in 30% sodium hypochlorite solution. The rhizomes were washed again in sterilized distilled water.

Water decoction procedure:-
The rhizomes of *Zingiber zerumbet* were ground gently using a mortar and pestle. The ground rhizomes were added into 300 ml of distilled water and the extract was boiled into half. Once it is almost 150ml, and the crude extract was filtered using a filter and syringe

Methanolic Extraction of Zingiber zerumbet:-
The Methanolic Extract of *Zingiber zerumbet* was prepared by macerating oven-dried (50^0C) powdered rhizomes (1.2 kg) of *Zingiber zerumbet* in 80% methanol in a ratio of 1:20 (w/v) for 48 h. The extract was agitated using a shaker for three to four days. The supernatant was collected, filtered and evaporated to dryness under reduced pressure (50^0C) yielding approximately 21.0 g of the crude dried extract. The crude dried extract was stored at -20^0C prior to use and was dissolved in normal saline (0.9% NaCl) immediately before administration (Zacharia ZA *et al*., 2010).

Preparation of the mixture of tobacco decoction and Zingiber Zerumbet:-
This mixture contained tobacco decoction- 1500ml, Neem Oil – 1000ml, Soap powder- 50 G.M.S., methanolic extract of *Zingiber zerumbet* – 9000ml. Neem oil- *Tobacco decoction* was made by mixing the decoction of tobacco, neem oil, soap powder and methanolic extract of *Zingiber zerumbet*.

Detection of antifungal properties of *Zingiber zerumbet* against phytopathogenic fungi:-
Dual culture technique:-
Antifungal properties of *Zingiber zerumbet* was evaluated against each phyto pathogenic fungi namely *Fusarium oxysporum*, *Pithium* sp., *Colletotrichum gloeosporioides*, *Corynespora cassicola*, *Rhizopus* species and *Aspergillus niger* by dual culture technique as described by Morton and trouble (1955). The experiment was done using water decoction of *Zingiber zerumbet* as well as the methanolic extract. To detect the antifungal effect of *Zingiber Zerumbet*, the extract and test fungi were placed opposite to each other of a SDA plate at equal distance from the periphery. The experiment was done in duplicate. In control plates, a disc of test fungi was inoculated at the centre of the media. Inoculated plates were incubated at 27 ± 3^0 C until the end of the incubation period (7 days after inoculation). At the end of second, fourth and sixth days of incubation period, radial growth of the colony of pathogen was measured. Percentage of inhibition was calculated by following the equation suggested by Fokkema and Shearer (1973).

Percent inhibition = ($R_1 – R_2/ R_1$) x 100 (R_1- radius away from the antagonist, R_2 - radius in direction of the antagonist)

Greenhouse experiment :-
Compatibility of *Zingiber zerumbet* extract with tobacco decoction was assessed under green house conditions by performing pot culture experiment with tomato plants. In greenhouse experiment seedlings were exposed to five different conditions. 2 controls and 3 treated groups. In control I seedlings were moistened with sterile distilled water and in control II seedlings were moistened with *Fusarium oxysporum* suspension containing approximately $4x10^4$ spores. Seedlings sprayed with the decoction of tobacco was taken as group I and seedlings treated with the combination of tobacco decoction and methanolic extract of *Zingiber Zerumbet* and challenged with *Fusarium oxysporum* species as group II and sapling was sprayed with the methanolic extract of *Zingiber zerumbet* alone was taken as group III.

Preparation of Spore Suspension:-
Spore suspension of *Fusarium oxysporum* was prepared according to the method of Ming Ming Yang *et al*, 2012 with some modifications. 8 agar blocks of actively growing *Fusarium oxysporum* was inoculated in to an Erlenmeyer flask containing 20 ml of potato dextrose broth. The flask was incubated at 27 ± 3^0C for 3 days. The flask was then chilled at 4^0C for 30 min to induce the release of spores and then incubated at 25^0 C for 30 minutes. Then the culture was filtered through cheese cloth to remove the agar blocks and attached mycelia. The resulting spore suspension was quantified using a hemocytometer.

Treatment Summary:-
All the pots used in this test were sterilized with 4% sodium hypochlorite for 15 minutes. Five saplings per pot were used, thereby, making a total of 25 plants per treatment. Saplings were maintained under five different conditions, 2 controls and 3 treated groups. In control 1 seedlings were moistened with sterile distilled water and in control II seedlings were moistened with *Fusarium oxysporum* suspension containing approximately $4x10^4$ spores were used. Seedlings moistened with tobacco decoction were taken as group I, seedlings treated with tobacco decoction incorporated with *Zingiber zerumbet* extract and later challenged with *Fusarium oxysporum* spores were group II and saplings exposed to *Zingiber zerumbet* extract alone were considered as group III.

Ten days old saplings of tomato with two or three leaves were transplanted from trays to 15cm diameter pots filled with 15 kg soil (presterilized for three consecutive days) and the green house condition was set at $28 \pm 2^{\circ}$C with 70% humidity. Pots were watered once in two days with sterile distilled water for 15 days. Two days later of planting, 15ml of tobacco decoction was sprayed onto the soil near the roots of group I and 15 ml of tobacco decoction and *Zingiber zerumbet* extract was sprayed onto group II respectively. After four days, 5ml of the spore suspension of *Fusarium oxysporum* containing $4x10^4$ spores were sprayed on the soil near the roots of group II. And 15ml of methanolic extract of *Zingiber zerumbet* was sprayed on to the roots of the plants in group III.

Plants were harvested 15 days after transplantation and the effect of *Zingiber Zerumbet* and *Tobacco decoction* on different growth parameter was recorded. Differences in root length (cm), shoot length (cm), number of leaves, number of roots, wet mass (gm), and dry mass (gm) were measured as the indicative of plant growth. And the ability of the plant to tolerate the tomato wilt disease was also observed. (modified method of Ming-Ming Yang *et al*., 2012)

Result and Discussion:-

At present, there is an increasing interest all over the world in herbal medicines followed by the extensive experimental investigation into the pharmacological properties of the bioactive components present in them. The *Zingiberaceae* plants, in particular have received much attention since they can produce so many complex compounds that can be used in flavouring and seasoning, cosmetics and as antioxidant and antimicrobial agents.

Dual culture technique was done to test the antifungal activity of water decoction as well as the methanolic extract of *Zingiber zerumbet* against *Fusarium oxysporum, Rhizopus* sp., *Colletotrichum gloeosporioides* ,*Corynespora cassiccola, Pithium species and Aspergillus niger*. From the dual culture experiment using water decoction of *Zingiber zerumbet* it was found that the action of water decoction of *Zingiber zerumbet* is almost negligible against the above said strains of phytopathogenic fungi.

Out of the six fungal strains tested, *Fusarium oxysporum* is the most highly inhibited fungal strain by the methanolic extract of *Zingiber zerumbet* with 64.72 % while *Corenyspora* and *Pithium* species are comparatively lesser inhibited with 50.26% and 50% respectively by the methanolic extract of *Zingiber zerumbet*. Methanolic extract of *Zingiber zerumbet* does not have any inhibitory effect at all against fungal strains like *Rhizopus, Colletotrichum* species and *Aspergillus niger*. From this experiment it may be concluded that the methanolic extract of *Zingiber zerumbet* has potent antifungal property against the above said strains of fungi **(table1)**. The antibacterial activity of the methanolic extract of *Zingiber zerumbet* was also reported against the leading bacterial strains such as *Staphylococcus aureus* and *Vibrio parahemolyticus* (Golam Kader *et al.*, 2011).

Effect of *Zingiber zerumbet* on the growth of tomato plantlets under greenhouse conditions:-
Six parameters of plant growth were taken into consideration. The parameters under consideration were the following; root length, shoot length, number of roots, number of leaves, wet mass and dry mass.

In order to detect the impact of the methanolic *Zingiber zerumbet* extract on the plant, and the compatibility of the extract with the tobacco decoction various growth parameters were analysed. Ten days old saplings were grown under different conditions **(Table 2)**.

By comparing both control I and group I, it was found that there were no marked differences between the plants belonging to these two groups. Using dry weight as a criterion for plant growth tend to be more trustworthy in these two groups of plants, because dry weight excludes the fluctuating water content in plants. The result showed the intact and healthy condition of plants treated with tobacco decoction.

By comparing both control II and group II, it was observed that there were marked differences between the two in all the six parameters of growth analysed. There was a steady increase in the root length, shoot length, wet mass and dry mass of the plantlets which were treated with the compatibility of the methanolic extract of *Zingiber zerumbet* and tobacco decoction.

By comparing both group I and group III we realized that the plants, treated with the methanolic extract of *Zingiber zerumbet* were healthier than those plants treated with the traditional biocontrol agent tobacco decoction because they have more number of roots and leaves. Moreover, the length of the roots were also found to be more in these saplings.

Saplings treated with the tobacco decoction incorperated with *Zingiber zerumbet* extract was found to be healthier and free from infectious diseases and pests. The mixture of these two extracts can be supposed to be rich in phytochemical components with fungicidal as well as insecticidal properties. *Fusarium oxysporum* is the most leading tomato plant fungal pathogen causing wilt disease and was highly inhibited by the methanolic *Zingiber zerumbet* extract with 64.72% inhibition. As far as the economic importance of possible use of this mixture as a biocontrol agent is concerned, it is biodegradable, cost effective, easily available and its processing is rather cheap. Moreover being natural, these may be no hazard to the environment as a whole.

Table 1. Inhibition of phytopathogenic fungi by the methanolic extract of *Zingiber zerumbet*

Phytopathogenic fungi	Percentage of Inhibition
Fusarium oxysporum	64.72 ± 0.31
Corynespora cassicola	50.26 ± 0.4
Pithium sp	50

Table 2. Use of biocontrol agent containing extract of *Zingiber zerumbet* and tobacco decoction for tomato wilt

Parameters	Control I	Group I	Control II	Group II	Group III
RL(cm)	6 ± 0.1	6.5± 2.24	4.5±0.1	7.5 ± 1.2	7.0 ± 2.1
SL(cm)	15± 0.3	13.2 ± 0.5	12 ±0.4	14.2 ±0.9	14.0 ± 1.2
WM(gm)	2.53 ± 0.5	2.25±1.5	0.81±0.1	2.53 ±1.5	2.36 ±1.5
DM(gm)	1.79±0.9	2.0 ±1.2	0.74 ±0.5	2.38 ±1.3	2.03 ± 1.2

Control I- Distilled water only, Control II- *Fusarium* spore suspension only, group I- Tobacco decoction only, group II- tobacco decoction and methanolic extract of *Zingiber zerumbet* and challenged with *Fusarium* spores, group III- Methanolic Extract of *Zingiber zerumbet* only, RL- Root length in cm, SL- Shoot length in cm, WM-Wet Mass in gm, DM- Dry Mass in gm.

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