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RESEARCH ARTICLE

EFFICACY OF CERTAIN PLANT EXTRACTS THAN FUNGICIDES AGAINST FUSARIUM SP.AFF.F.SEMITECTUM BERK & RAVENEL CAUSING LEAVE WILT IN KACHAI LEMON (CITRUS JAMBHERI LUSH)

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Abstract

The efficiency of different natural plant extracts and chemical fungicides were conducted to determine their effects on *Fusarium* sp.aff.*F.semitectum* causing leave wilt of *Citrus jambheri* (Kachai lemon). In vitro studies to assess the antifungal activity of 10 plant extracts were carried out and the results revealed that plant extract had a strong antifungal property with significant inhibition in the growth of tested fungus. Among the phytoextracts, *Curcuma longa* was found to be most effective with 95.32 % to inhibit the growth of the fungus. Different concentrations of chemical fungicides were also studied on test fungus and were found more efficient than the natural compounds. Thus, the plant extracts can be used as a sustainable alternative fungicide to control pathogenic fungus like *Fusarium semitectum* thus reducing the dependence on the synthetic fungicides.

Introduction:-

The satisfactory control of the fungal diseases by using various chemicals has been documented in the literature (Choulwar et al., 1988, Maheshwari at al, .1991, Abdul et al., 1995). However, global concern for protection of the environment has led researchers to investigate the use of natural flora as one of the sources for crop protection (Wijewardane et al,. 2009).Different approaches may be used to prevent, mitigate or control plant diseases. Citrus, a tropical and sub-tropical crop belong to the family Rutaceae of the tribe citrae. Being a fruit crop of international importance, citrus is grown commercially in almost every country in different agro-climatic conditions (Naqvi 2004). The southern slopes of Himalayan region, the entire North Eastern states of India and adjacent China are considered as the original homeland of citrus (Mukhopadhyay and Thapa 2001, Gmitter et al., 1990). Citrus has a tremendous economical, social and cultural impact in our society. In spite of the high demand of citrus fruits, its production level is low due to pest and diseases. Citrus is attacked by a number of fungal, bacterial and viral diseases. Among these, fungal diseases are of great importance and cause huge economic loses up to 10 to 30 percent (IITA, 2003, NIHORT, 2003). The mandarin cultivation in Darjeeling has shown a massive decline due to various pathological, entomological and nutritional stresses (Mukhopadhyay et al. 1996).

Plant fungal pathogens are frequently found as one of the limiting factors for crop production. More than 10,000 species of fungi can cause disease in plants. Rough lemon is attacked by various fungal pathogens but *Fusarium* is of prime importance. Genus *Fusarium* occurs widely in nature as saprophytes in soils and decaying vegetables that have commonly associated with different varieties of citrus and can caused serious diseases. Some species are plant

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parasites where specialized pathotypes cause stem rot, ear diseases, vascular wilts, fruit rot and damping off diseases (Booth, 1971, Abd-Elgawad et al., 2010). The success of crop production depends on the ability of the farmers to overcome challenges to maintain functional and profitable farms. One of the most important challenges is preventing and controlling pests and diseases problems, which can partly or completely ruin agricultural crops (Suprapta, 2012). Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crops productivity and quality. Besides the development of resistance in pathogens against fungicides, environmental pollution and residual hazardous effects of fungicides on non target hosts, the control of pathogens by using the fungicides is the most common and instant means (Madhu, 2017). Drawbacks of synthetic chemical methods have increased interest in developing further alternatives control measures particularly those that are environmentally social and biodegradable (Sharma and Tripathi, 2009). Thus, replacement of synthetic fungicides by natural products particularly of plant origin, which are non-toxic and specific in their action, is gaining considerable attention. The present studies were therefore, made to assess the efficacy of phytoextracts and fungicides against the growth of *Fusarium* sp.aff.*F.semitectum* which caused leave wilt in Kachai lemon which is also accorded **Geographical Indication (GI)** tag recently.

Materials and Methods:-

Sample collection:-

Infected leaves of lemon (*Citrus jambheri*) were obtained from the Kachai lemon orchard. All the samples collected were placed in a sterile polythene bags with appropriate labeling and brought to the laboratory.

Isolation of fungi:-

The collected samples were used for the isolation of fungi by usual isolation procedure (Ricker and Ricker, 1936). The infected or disease leaves were washed in sterile water and cut into small pieces of 4-5mm length by using sterilize scalpel. The cut pieces were surface sterilized in 0.1% Mercuric chloride solution for 30 seconds. The sterilized pieces were then rinsed twice with distilled water and transferred on to filter paper in petriplates for drying. Then sterilized pieces were plated in petriplates containing Potato dextrose agar (PDA) medium (3 pieces of specimen in one petriplate) amended with 100 ppm of streptomycin sulphate. The plates were incubated at 28℃ $\pm$ 1 for 3-4 days. The fungi which colonized those pieces was purified and identified on the basis of macro-morphological and micro-morphological characteristics after consulting the relevant literature (Barnett and Hunter 1972)

Pathogenicity test:-

The isolates of *Fusarium semitectum* were tested in the detached lemon leaf assays. Completely randomized design (CRD) with 3 replications was used for the experiment. Each mycelia plug (5mm diameter) was taken from young fungal colony of the growing pathogen by sterilized cork borer and artificially inoculated onto wounded leaves. A needle was used to wound the leaves before placing 5mm diameter of mycelia disc from the isolate. The inoculated leaves were incubated in moist chamber (a moist tissue paper in Petridish) at room temperature (28˚- 30˚C) for 5-7 days. The non inoculated leaves were treated with sterile agar disc and served as control. After 72 hours, the inoculated leaves were observed for symptom development. The causal agents were re- isolated from the infected culture leave and compared with the original isolates. This experiment was replicated three times.

Evaluation of the effect of phytoextracts on the growth of fungal pathogen:-

Ten plants (*Centella asiatica, Tajeta petula, Alllium tuberosum, Justicia adhatoda, Azaderachta indica juss, Ageratum conyzoide,Solanum anguivii, Zingiber officinale, Curcuma longa* and *Mentha spicata*) were selected to estimate the antimycotic behaviour against *Fusarium* sp.aff.*F.semitectum* by poison food technique (Nene and Thapliyal, 1979) at four different concentrations viz. 5%, 10%, 15%, and 25% .Fresh plant parts of 25g from each plant were washed thoroughly 2-3 times with tap water and then again with sterilized distilled water. The surface sterilization was done finally with 90% ethanol. The plant materials were crushed in 100 ml sterile distilled water. The macerate was filtered through double- layered cheese cloth and centrifuge at 3500 rpm for 20 minutes. The supernatant was filtered through Whatman No.1 filter paper. Extract (75%) thus obtained was utilized for further experiments. The aqueous extracts were sterilized at 121˚C for 15 minutes prior to use. Required amount of stock solution of each plant extract was mixed thoroughly in molten PDA to get desired concentration just before pouring in sterilized Petriplate. Each plate was inoculated with 5mm disc of mycelia bit taken from 7 days old culture of *F. Semitectum* and incubated at 28˚±1℃. Each treatment was replicated thrice and the medium without fungicide served as control.

Evaluation of fungicides:-

Fungal toxicity effect of four systemic fungicide viz., Carbendazim 50% W.P, Thiophenate Methyl 70 % WP, Captan 50% WP, and Mancozeb 75% WP were screened in vitro by following poisoned food technique method (Nene and Thapliyal, 1979) against *Fusarium semitectum* at different concentration viz., 50 ppm, 75 ppm and100 ppm respectively. Required quantities of individual fungicides were added separately to sterilize PDA medium so as to get the desired concentration of the fungicides. After mixing thoroughly, 20ml of this mixture was poured into sterile Petriplate (9 cm diameter).Mycelial disc of 5mm size from actively growing culture of the test pathogen were cut by a sterile cork borer and one such disc was placed at the centre of each agar plate and incubated at 28˚±1℃ till the mycelia growth in the control reaches a maximum growth (120 hrs).Each treatment was replicated thrice. The medium without fungicide served as control. The diameter of the colonies was measured and average values compared with control were taken as a measure of fungal toxicity. Growth inhibition (%) of test fungal was determined by using the formula (Pani and Patra, 1997):

$$\textbf{Growth inhibition percentage (\%)} = \frac{\textbf{Control} - \textbf{Treatment}}{\textbf{Control}} \times 100$$

Statiscal analysis:-

Data obtained were subjected to statistical analysis following the method of variance described by Gomez and Gomez (1984). ANOVA was performed on the data and least significant difference (LSD) at 5 % level was calculated to determine significant differences between treatments.

Result and Discussion:-

The results revealed that plant extracts had a strong antifungal activity with significant inhibition on the growth of the tested fungi. Inhibitory effects of the selected phytoextracts on the radial growth of *Fusarium* sp.aff.*F.semitectum* were found to be different in all the concentration of ten phytoextracts. Among the phytoextracts, extracts of *Curcuma longa* were found to have the highest inhibitory effect (50.24%,53.45%, 55.69% and 59.39% at 5%, 10%,15% and 20%) followed by *Ageratum conizoides* (14.92%,33.7%,49.27% and 52,96% at 5%, 10%,15% and 20%) respectively. A possible explanation for the effective inhibition of the mycelia growth of the above said pathogens by different plant extracts might be due to the presence of antifungal compounds present in the leaf extracts (Gosh et al., 2002; Kagale et al., 2004). Mycelial growth of various species of *Fusarium* was inhibited by the plant extracts of *Azadirachta indica* (Eswaramonthy et al, 1989), *Allium sativum* and *Sapertus trifoliate* (Gohil and Vala, 1996). On the other hand, the chemical fungicide was more efficient than natural compounds. All the fungicides inhibited the radial growth of the fungi amended with different concentration of fungicides but showed variations in extend of inhibition. Application of Streptocycline after pruning or spraying in combination with copper oxychloride was found to be effective in reducing the citrus bacterial canker pathogen on acid lime (*Citrus aurantifolia*) under field condition in gangetic plains of West Bengal (Hansda et al.2013).Among the four test fungicides, Carbendazim was most effective showing100% inhibition on mycelial growth of *F.semitectum* followed by Thiophenate Methyl, Captan and Mancozeb the least effective. The present study revealed the application of fungicide was more effective than the application of phytoextracts. However, the disease control by biocontrol agents reduced problems such as toxic to non target species, lower risk of fungicide resistance and lower environmental negative impact. The rationale for exploiting plants for their antibiotic capabilities stems from the ability of plants to produce a wide array of secondary metabolites present a large and relatively untapped source of antifungal drugs (Amoo et al. 2009; Sharma and Tripathi, 2009).

Table 1:- In vitro evaluation on effect of different phytoextracts on the linear mycelial growth of *Fusarium* sp.aff.*F.semitectum*.

Phytoextracts	**Radial growth diameter (mm) at 120 hrs**				
	Control(mm)	**5%**	**10%**	**15%**	**20%**
Centella asiatica	62.3	53 ± 0.45	51.3 ± 0.40	51 ± 0.36	37 ± 0.43
Tajeta petula	62.3	55 ± 0.1	47.3 ± 0.15	42.3 ± 0.25	40.6 ± 0.40
Alllium tuberosum	62.3	58.6 ± 0.15	54.3 ± 0.25	53.6 ± 0.25	50.6 ± 0.37
Justicia adhatoda	62.3	61.3 ± 0.25	59.3 ± 0.23	56.3 ± 0.32	48 ± 0.1
Azaderachta indica	62.3	55.6 ± 0.49	53.6 ± 0.05	49.3 ± 0.11	44 ± 0.36
Ageratum conyzoide	62.3	53 ± 0.26	41.3 ± 0.30	31.6 ± 0.05	29.3 ± 0.45
Solanum anguivii	62.3	55.3 ± 0.17	52.5 ± 0.22	52.6 ± 0.25	51.1 ±0.16
Zingiber officinale	62.3	43 ± 0.15	39.3 ± 0.15	38.3 ± 0.15	35.3 ± 0.35

Curcuma longa	62.3	31 ± 0.3	29 ± 0.3	27.6 ± 0.15	25.3 ± 0.05
Mentha spicata	62.3	58.6 ± 0.15	57.6 ± 0.15	55.3 ± 0.05	53 ± 0.26
Mean ± SE C.D (p=0.05)		0.22 0.46	0.19 0.39	0.18 0.37	0.27 0.56

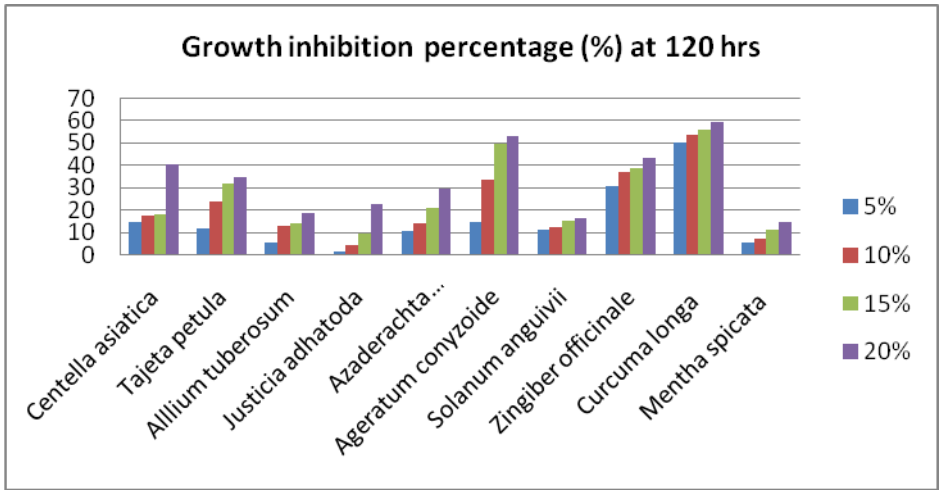

Fig. 2:-In vitro evaluation on the growth inhibition (%) of *Fusarium* sp.aff.*F.semitectum* by ten phytoextracts.

Table 2:-In vitro evaluation of effects of different fungicides on the linear mycelium growth of *Fusarium* sp.aff.*F.semitectum*

Fungicides	**Radial growth diameter (mm) at 120 hrs**		
	50 ppm	**75 ppm**	**100 ppm**
Carbendazim 50% W.P	0 ± 0	0 ± 0	0 ± 0
Thiophenate Methyl 70% W.P	12 ± 0.55	11.6 ±0.25	0 ± 0
Captan 50% W.P	12.6 ± 0.05	12 ± 0.1	11.6 ± 0.05
Mancozeb 75% W.P	15.6 ± 0.30	13.6 ±0.25	11.9 ± 0.15
Mean ± SE	0.29	0.15	0.065
C.D (p = 0.05)	0.58	0.31	0.13

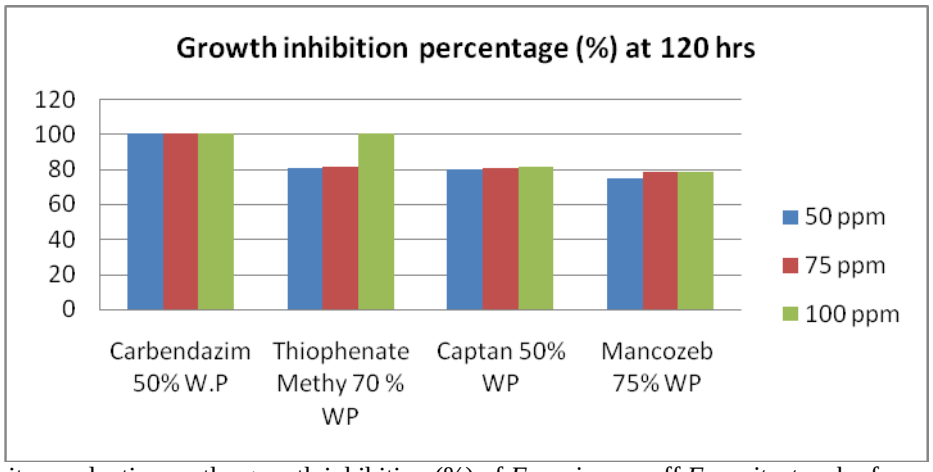

Fig 2:- In vitro evaluation on the growth inhibition (%) of *Fusarium* sp.aff.*F.semitectum* by four commercial fungicides

Conclusion:-

The result of the present study revealed that extracts of *Centella asiatica, Tajeta petula, Alllium tuberosum, Justicia adhatoda, Azaderachta indica juss, Ageratum conyzoide, Solanum anguivii, Zingiber officinale, Curcuma longa* and

Mentha spicata have been emerged as sustainable alternative to the use of chemical fungicide and can be used as an eco-friendly fungicides. Further work is required to increase the efficacy of these plant extracts in field condition and also to determine the biologically active ingredient present in extracts as well as its mode of action.

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