Isolation And Identification Of Fungi From Some Leafy Vegetables In Latur District

[1]Dr. V. S. Chatage
[1]Assistant Professor
[1]kai. Rasika Mahavidyalaya, Deoni

Abstract **- The present investigation was carried to study the different fungal sp. present in various leafy vegetables. Six fungi, which caused leafy vegetables in latur district area, were isolated from various samples of different leafy vegetables in flied, Isolated from diseased leafy vegetables on PDA media. The isolated fungi were identified as Alternaria tenuissima, Fusarium proliferatum, Alternaria spinaciae, Fusarium oxysporum f. sp. Spinaciae, Alternaria alternata, and Phytophthora colocasiae on the basis of their microscopic characteristics. Finally, the pathogenicity tests showed that all the fungi isolated were pathogenic to all the leafy Vegetables**

keywords **- leafy Vegetables, Fungi and Potato Dextrose Agar (PDA) media and pathogenicity tests.**

I. INTRODUCTION

Vegetables are increasingly becoming important as produce for domestic and export markets. They have a great potential to improve the nutrition and there by health of consumers as most are good sources of vitamins, minerals and proteins needed for the proper functioning and development of the human body [1].Vegetable however, have serious challenges to their existence and these include changes in climatic condition, pests, inadequate rainfall and fungal attack [2]. Post harvest losses of vegetables are particularly high in the tropics and may be in the order of 25% and even higher for more perishable produce [3]. Losses in fruits and vegetables are more serious in developing countries than the developed ones. In Ghana, it is estimated that about 20% to 30% of fresh food products including vegetables harvested each year never reach the final consumer in the market because they are either lost or damaged during the various stages of the distribution chain [4].

Vegetables are more susceptible to insects pests and diseases due to their tenderness and softness as compared to other crops and virtual absence of resistance characters because of intensive hybrid cultivation [5].The native value of healthy vegetables are altered because of fungal attack and sometimes fungi produce certain mycotoxin in them and make them unsuitable for human consumption. Thus the present study was aimed to evaluate the Fungal spp. in laboratory conditions identify and isolate pathogencity tests the fungal associated with their leafy vegetable.

II. MATERIALS AND METHOD

i) Isolation of pathogens

Flied presented leafy vegetables infected parts of selected vegetables viz. *Rumex acetosa L.*, *Spinacea oleracea L.*,, *Trigonella foneum-graecum*, and *Colocasia esculanta* were brought into the laboratory and cut into small pieces (1-2 mm) by sterilized blade then surface sterilized with 1% mercuric chloride ($HgCl_2$) for 1 min. The pieces were washed with sterilized distilled water thrice. The pieces were incorporated with Potato Dextrose Agar medium (PDA) kept at 27±2^{0}C and pathogens were isolated and identified by manuals [6;7, and 8]

The fungal isolates were identified on the basis of colonial, morphological characteristics and micrometry. The fungal colonies identified on the basis of microscopic examination were purified. The morphology of isolated fungi was studied by Lactophenol cotton blue staining [9].

ii) Pathogenicity test: Pathogenicity (on leaves).

For pathogenicity test isolates were grown on PDA for 7 days inoculation was done using detached surface sterilized on leaves. A single drop (5µl) of spore suspension (1x10$_3$conidia/ml) was placed on each leaves. Leaves were incubated in humid growth chamber (80-90% relative humidity) for intensity with a photoperiod of 12h. After 8 days, leaf spots similar to the original symptoms were developed on all tested leaves and root was consistently re-isolated fulfilling Koch's postulates [10]. Control leaves inoculated with sterilized distilled water remained symptomless.

Pathogenicity (on roots).

Pathogenicity test were conducted, using healthy leafy vegetable grown in the glass house. Two plants and a control were used for each of two replications. For each treated plants were sprayed with conidial suspension (ca.1×10$_5$conidia/ml) and maintained in a humid growth chamber for 24h in room temperature. Five days after inoculation white mycelial roots were infected. No control displayed symptoms and remained healthy in the tests. The fungus was reisolated and identical to the stock culture. Pure cultures were maintained on PDA slants.

Table 1: Cultural and microscopic characteristics of fungi isolated from different leafy vegetables.

Fungal isolate No.	Name of the Leafy Vegetables	Cultural characteristics	Reverse side of Colony	Microscopic characteristics	Fungal species identified
LVGf *1	**Chuka** *Rumex acetosa* (L.)	Green colonies with whitis peripheral . Concentric rings are formed. Radial growth of the fungus in culture was uniform.	Green	Conidiophores solitary or in groups, simple or branched less cylindrical, septate pale or mid pale brown smooth with 1 or several scars up to 115 µm long 4-6 µm thick. Beak, generally 4-7 transverse and 0-6 longitudinal septa. Total length of spores is 22-95 (54) µm, 8-19 (13.8) µm thick in the broadest part and beak 2-4 µm.	**1)***Alternaria tenuissima*
LVGf *2		The abundant aerial mycelium initially was white and later became purple violet. Colonies were fast growing, hyphae were septate. Conidiophores were short, simple and branched.	Hyaline	Microconidia were abundant and produced on mono and polyphialids, single celled, oval to clubshaped size 7.0-22.5~3.5µm. Macroconidia were slightly sickle shaped to straight, with 2-5 septa and measured 43-65×3.3-5.0µm. Chlamydospores were absent.	**2)***Fusarium proliferatum*
LVGf *3	**Spinach** *Spinacea oleracea* (L.)	Blackish white ash colonies with whitish peripheral concentric rings are formed.	Black	Hyphae short, septate, olivaceous, conidia elongate, clavate, 6-10 septate, yellowish to olivaceous, 80-120×12-14 µm.	**3)***Alternaria spinaciae*
LVGf *4		Colonies cottony whitish soft texture. becoming pink in colour on maturity, cottony, fast growing	Hyaline	Conidia when born in sporodichia typically broader toward the apex and usually abruptly constricted at the apex, pedicellate, mostly 3-septate 35×5-25(27-43×4.8-6.3) µm;1-septate 20×4.8(14-24×4.4-5.0) µm;0 septate 8×3.2(6-11×3-4.8) µm.	**4)***Fusarium oxysporum f*.sp.*spinaciae.*
LVGf *5	**Fenugreek** (*Trigonella foneum-graecum*)	Colonies usually black conidia formed in long, often branched chain.	Black	Overall length 20-63(37) µm, 9-18(13) µm thick in the broadest part; beak pale, 2-5µm thick.	**5)***Alternaria alternata* (Fr.) Keissler

LVGf *6	**Colocasia** (*Colocasia esculanta* L.)	Mycelium branched and coenocytic, sporangia on specialized hyphal sporangiophores which are branched and with indeterminate growth.	Hyaline	Zoospores reniform, laterally biflagelated. Sporangia were ovoid, hyaline, papillate, caducous, 30 to 60 × 17 to 28 µm, and pedicels were 3.5 to 10 µm long.	**6)** *Phytophthora colocasiae* Rac.

LVGf*- LVG-leafy Vegetable, f*-fungi.

III. RESULTS AND DISCUSSION

Six fungal species are isolated from various leafy vegetables. Table 1 shows the viable count of fungal isolates (LVGf*1 to LVGf*6) on PDA plates. Table 1 represents fungal species identified on the basis of reverse side of colony, cultural and microscopic characteristics. The fungal isolate LVGf*1 to LVGf*6 was present in all tested healthy leafy vegetables. The vegetables associated fungi isolated from various samples of different leafy vegetables identified on the basis of their macroscopic and microscopic characteristics are *Alternaria tenuissima* (LVGf*1), *Fusarium proliferatum* (LVGf*2), *Alternaria spinaciae* (LVGf*3), *Fusarium oxysporum f*.sp.*spinaciae* (LVGf*4), *Alternaria alternata* (Fr.) Keissler (LVGf*5) and *Phytophthora colocasiae* Rac. (LVGf*6) fungi were isolated.

Rumex acetosa Isolated fungus was identified as *Alternaria tenuissima* (Fries) Wiltshire [7; and 8]. There are reports *Alternaria tenuissima* of causing disease on blueberry & pepper in China, but there is no previous report of the pathogen on sorrel plants [11] and [12]. *Rumex acetosa* Leaf spots similar to the original symptoms were developed on all tested leaves and *A. tenuissima* was consistently re-isolated fulfilling Koch's postulates [13]. In order to prevent it, plants should be set apart for good air circulation and, when watering, the plant's foliage should not get wet. To help avoid soil borne diseases, such as *Rhizoctonia*, *Pythium* or *Fusarium*, the plantings should be rotated each year; in other words, spinach should not be sowed in the same row or bed every year [14]. *C. esculenta* farmers ranked diseases as the major constraint halting the cultivation of *C. esculenta.* Diseases observed on the farmers' fields affected mainly the shoot. This agrees with observation by [15]. *C. esculenta* leaf blight disease has been thought to be caused by *Phytophthora colocasiae* [16; 17 and 18]. [19] Published the only documentary evidence of *C. esculenta* disease in Ghana caused by *Cladosporium colocasiae* and remarked on critical suppression of symptom development with Thiophanate methyl. The present study did not review *Phytophthora colocasiae* in *C. esculenta* fields. Contrarily, *Cladosporium colocasiae* produced leaf spot symptoms on upland *C. esculenta.*

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