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Investigation of Phyllospheric Mycoflora of Chili from western Vidarbha, Maharashtra

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ABSTRACT

In the present research work phyllosphere mycoflora of Chili, was undertaken, to investigate different types of fungal mycoflora. The leaf, stem and fruit samples were collected from various field of Amravati and Akola region at the intervals of 15 days starting from seedling state up to its harvesting. The diseases samples were collected in all the stages of plant i.e. seedling, foliage, flowering and fruit-formation stage. The samples from plants were brought into laboratory in separate sterilized polythene bags for isolation of all possible phyllosphere mycoflora. The infected plant parts which were collected different stages of disease development and all the samples were carefully preserved in the form of herbarium to study the etiology of diseases. The symptoms of diseases were studied in the beginning as well as severity of infection. Phyllosphere isolation were carried out by different methods such as serial dilution, leaf impression, leaf washing methods, etc. The different types of fungal pathogens were also isolated by cutting small fragments of aerial diseased parts of plants from junction of infected and healthy parts. From the phyllosphere study it was concluded that mainly Deuteromycetean fungi were found to be associated with various parts viz. leaf, stem and fruit. The fungal isolated were identified with the help of available literature and stock cultures.

Keywords: - Chili, Pathogen, Symptoms, Morphology, Phyllospher, Mycoflora

I. INTRODUCTION

Various surveys and regional surveys were made on mycoflora of Indian subcontinent including fungi of Bombay, Wangikar and Ballal (1984). The present data revealed that mostly Deuteromyces fungi were associated not only with the leaves of various wild plants but also ornamental and crops of economic value. It has been reported that genera belonging to the from order moniliales such as Fusarium, Curvularia lunata, Helminthosporium sp., Bipolaris sp., Alternaria, Botrytis, Cladosporium, ; those belonging to the form order Melanconiales such as Colletotrichum, Glommerella, Melanconium, Polnema and of the order Sphaeropsidales like Phoma, Phyllosticta, Coelophoma, Macrophoma, Macrophomina, Phomposis, Sphaeropsis, Ascochyta, Botryodiplodia have worldwide distribution. From the available literature it is revealed that the distribution of

leaf spot fungi could not be restricted by ecological conditions and geographical barriers. Among all plant pathogens, fungi are the major disease causing agents and can be responsible for about 90% of agricultural yield loss (Maninegalai et al., 2011). It has been reported that various genera of fungal pathogen causing diseases on vegetables like brinjal are leaf spot and frost rot caused by Phomopsis vexans, colletotrichum fruit rot caused by Colletotrichum melogenae, brinjal wilt caused by Verticillium albo-atrum, etc.

Macrophomina phaseolina is an anamorphic fungus in the ascomycete family Botryosphaeriaceae (Crous et al., 2006). Macrophomina is primarily soil and seed-borne fungal pathogen that incites disease by producing microsclerotia or pycnidia (Pun et al., 1998). With a wide host range of approximately 500 species in 75 plant families, the pathogen exhibits heterogeneous host specificity i.e., the ability to infect monocots as well as dicots and exhibits non-uniform distribution in the soil (Mayek-Perez et al., 2001). M. phaseolina consists of only one species. In spite of being a mono-specific genus, M. phaseolina exhibits a high degree of morphological (Mayek-Perez et al., 1997), pathogenic (Su et al., 2001), it has worldwide distribution. From the available literature it is revealed that the distribution of the leaf, stem and fruit surface could not be restricted by ecological condition. In present investigation, the study of phyllosphere mycoflora was undertaken to screen the diseases causing fungi of the vegetable leaf, stem and fruit surface.

II. MATRIAL AND METHODS

Collection of materials and isolation of fungi:

A regular survey of phyllosphere fungi of vegetable from different region viz. Amravati and Akola region was made during the month of January 2013-January 2015. Various fungi causing diseases of vegetables were collected. Isolation of fungi was made by cutting the small pieces of leaves from the junction of healthy and infected region. Infected pieces were surface sterilized by sodium hypochloride and transferred aseptically to sterilized slant in culture tubes. The slant was prepared from Ashtana and Hawker, s medium 'A'.

Study of phyllosphere mycoflora by serial dilution plate method

Serial dilution plate method is one of the common method adopted for study of phyllosphere microflora. Fresh healthy leaves of all ages were collected from Tomato, field in fresh sterile polythene bags and brought into the laboratory.

Study of phyllosphere mycoflora by leaf impression method

This method was useful for superficial fungal spores. At the time of isolation fresh leaf was taken and pressed from its dorsal surface momentarily against surface of petri plate at three places. Same leaves were placed from ventral surface against the agar surface in same way like the first. Same procedure was repeated for other leaf sample. Incubation of plates was carried out at 26oC in an inverted position for 7 days.

Leaf washing method

This method was established by and describe as standard washing method for isolation micro flora of leaf. The process involved the cutting of 3 mm diameters uniform discs at random from leaf surface sample and washing them in 2-3 changes of sterile water. Aliquots of the final washing were placed out with the tap water agar and incubated to determine the efficacy of washing process. The results were taken after 3 days of incubation.

Identification of isolate

The isolates were identified from available literature Illustrated genera of imperfect fungi by Barnett and Hunter (1972); Morphological and taxonomical studies of all the fungi isolated were carried out. During the studies of phyllosphere fungi vegetative and reproductive characters were recorded to known the species identification.

Pathogenicity test

Pathogenicity of the organism was confirmed by Koch's postulate method. In order to pathogenicity, perfectly healthy, vegetable leaves of similar size were selected and thoroughly washed with sterile water. The fungus was claimed as pathogen only after satisfying Koch's postulates. Wherever more than one organism was isolate from a single lesion, pathogenicity of each organism was confirmed separately. During the studies the author made specific attention on variation in symptoms, month wise observation, nature of agriculture practices. All the data were recorded at the time of disease survey.

III. OBSERVATION AND RESULTS

Diseases of Chili: -

1) Fulvia fulva

Disease sample: - Fruit

Name of disease: - Fulvia fruit spot

Causal agent: - Fulvia fulva

Symptoms of Disease: - Pale green, yellowish spots appear on the upper surface of fruit, an olive-green to tan, velvety fungal growth appears on the lower surface of the spots. Under favourable conditions (long periods of humidity greater than 85 percent), complete defoliation can occur.

Morphology of pathogen: - Colony is greenish-black colour, thread-like, velvate, conidiophores dark, erect, slender, usually simple, septate, long; conidia hyaline, borne singly at apex or produced in chain 1- Celled, bunched of conodia mostly globus or ovoid, fusoid, in dry basipetal chain. Conidiophores are 100-150 µm in length and 2-4 µm in width, the conodia has 2-4 µm in diameter area.

2) Alternaria solani

Diseases sample: - Fruit

Name of disease: - Fungal Fruit Rot

Causal agent: - Alternaria solani

Symptoms of Disease: - Symptoms first appear as water-soaked, gray lesions that collapse and darken. Lesions turn velvety as spores are produced. Infection generally occurs at growth cracks, injuries or at the blossom-end of fruit. Internal colonization of pepper fruit without external signs of infection can occur when flowers are infected, the cracks appearing on leaf spot. The Alternaria solani occurs commonly, causing the characteristic leaf spots with concentric rings. The spots are mostly irregular, 6-9 mm in diameter and may coalesce to cover large areas of the leaf blade. Severely affected leaves may drop also infects the fruits causing large deep-seated spots. The infected fruits turn yellow and drop off prematurely.

Morphology of pathogen: -Mycelium is septate, branched, light brown and dark brown. Conidiophores emerge through stomata and dark colored. Conidia are single celled, muriform, beaked and produced in chains. The conidia are with 6-8 transverse septa and a few longitudinal or oblique septa.

3) Alternaria alternata(Fr) C. Keissler,

Disease- sample: - Fruit

Name of disease: - Alternaria Rot

Causal agent: - Alternaria alternate

Symptoms of Disease: -Symptoms first appear as water-soaked, gray lesions that collapse and darken. Lesions turn velvety as spores are produced. Infection generally occurs at growth cracks, injuries or at the blossom-end of fruit. Internal colonization of pepper fruit without external signs of infection can occur when flowers are infected.

Morphology of pathogen: - The pure culture was isolated on 27 ± 2^0 C produed colonies, olivaceous, black-grey, creamish yellow to black. Conidiophores arising singly or in small groups, branched, straight or curved; Colonies 5.5-6.0 cm diameter after 3-5 days at 27^oC, reverse smooth, up to 40 µm long, 2 – 4 µm thick, with 1-2 or several apical conidia. Conidiaporospores often in branched chains, obclavate, ovoid, with a short conica or cylindrical beak, golden brown, up to eight transverse and several longitudinal, oblique septa, 25-65 µm long, 10 – 20 µm thick, beak pale, 2 – 5 µm thick.

4) Alternaria porri (Ellis)Cif.

Diseases sample: - Leaf

Name of disease: - Alternaria leaf spot

Causal agent: - Alternaria porri

Symptoms of Disease: - The disease causes characteristic leaf spots with concentric rings. The spots are mostly irregular and coalesce to cover large areas of the leaf blade. Severely affected leaves drop off.

Morphology of pathogen: - Alternaria porri colonies are rapid growing, cottony, and gray to black. The conidia develop in branching chains at the apex of the conidiophore, with the youngest conidium at the apex of each chain. The conidia are dematiaceous and muriform. The conidia are large breadth is 4-6 µm in diameter and their breadth is 40-70 µm in diameter.

5) Colletotrichum capsici (Syd.) Butl. Bisby

Disease sample: - Fruit

Name of disease: - Anthracnose fruit rot

Causal agent: - Colletotrichum capsicii

Symptoms of Disease: - Fruit lesions are the most important character of this disease. Fruit symptoms begin as water-soaked areas that turn tan or brown. Lesions may be small and circular, coalesce to cover large areas of the fruit. Under moist conditions, pink, salmon or orange masses of spores are formed, usually in concentric rings. Depending on the species present, black or brown flamentous structures may be visible in the lesion. Anthracnose can affect both green and ripe fruit, but symptoms are usually not visible until fruit ripen and turn red. Warm, wet weather generally favours infection and development of symptoms. Depending on the species of Colletotrichum present, optimal temperatures for infection range from 20° C to 27° C, free moisture is

necessary for infection. Fog and dew are conducive to disease development. Rain disseminates the pathogen's spores and often leads to severe losses, especially if fruit are wounded

Morphology of pathogen: -The isolates of C. capsici were obtained from the chili fruits infected with fruit rot. Each showed colony colour, pattern of growth on PDA. The isolate is white to light mouse grey, circular, mycelium with black coloured all over the colony growth. Whereas, isolate produced dark black to brown coloured circular flat mycelium with scattered and black coloured acervulus. The colony diameter after 5 days of incubation at 27±2°C was found. Conidia were hyaline, unicellular, fusiform curved with narrow ends. The average dimensions of conidia which possessed large oil globule in the centre, varied between the isolates in dark brown setae with several septations and pointed brown tips.

6) Colletotrichum acunatum

Disease sample: - Leaf

Name of disease: - Anthracnose leaf spot

Causal agent: - Colletotrichum acunatum

Symptoms of Disease: - Anthracnose affects all above-ground parts during any stage of growth. Seedling infection may be confined to cotyledons. Necrotic gray to brown spots may develops on leaves and stems. Fog and dew are conducive to disease development. Rain disseminates the pathogen's spores and often leads to severe losses, especially if fruit are wounded. These fungi can survive in infected seed and persist in leaf or stem lesions, in plant debris for long periods of time.

Morphology of pathogen: - Colonies are rapid growing, cottony, and gray to black with dark brown to black acervuli. The mycelium is branched, septat cottony thread. The acervuli are irregular in shape and consist of dark setae. Sometimes acervuli are also formed on the leaf. Acervuli are rounded, elongate, separate, superficial, erumpent, with conspicuous multicellular, dark seta, and 80-90 µm in diameter. Conidiophores are hyaline, single-celled, falcate, fusiform, spindle shaped, with acute apices, and measure 17-24 x 2.3-3.8 µm. Setae are brown with a dark swollen base and a pale rounded tip. Conidia single celled.

7) Cladosporium fulvum Link.

Disease sample: - Leaf

Name of disease: - Cladosporiumleaf spot

Causal agent: - Cladosporium fulvum

Symptoms of Disease: - The disease causes characteristic leaf spots. The spots are mostly irregular areas on the leaf. Severely affected leaves drop-off.

Morphology of pathogen: - The colony brown to black. Colonies are pale gray or grayish brown, thinly hairy on natural substrata cottony or loosely felted in culture. Hyphae creeping, conidiophores almost erect branced, and floccose, septate on the surface, often forming a turf, olive coloured conidia globose and ovate usually greenish terminal and then passed to the side. Cladosporium is characterized by erect, conidiophores with chains of conidia in tree-like heads. Conidiophores long, upright and branching at apex; conidia variable in shape oval, cylindrical or irregular and produced in chains, saprophytic or pathogenic. The lemon-shaped conidia which has well marked dark attachment scars and show considerable variation in size. The conidiophores are macronematous, straight or slightly flexuous, distinctly nodes, pale or mid-pale brown, smooth, up to 50 µm long or sometimes even longer in culture, 2-6 µm thick, with terminal and intercalary

swellings of 4-6 µm diameter. Conidia arise in terminal, in simple or branched chains. Conidia are cylindrical, rounded at the ends, ellipsoidal, subhyaline or pale olivaceous brown, smooth, 4-20 x 2-4 µm.

8) Alternaria sp.

Disease sample: - Leaf

Name of disease: -Alternaria leaf spot

Causal agent: - Alternaria sp.

Symptoms of Disease: - The disease causes characteristic leaf spots with concentric rings. The spots are mostly irregular and coalesce to cover large areas of the leaf blade. Severely affected leaves drop-off.

Morphology of pathogen: - Colonies usually brown to black, hyphae dark brown, branched. Hyphae are aseptate, 1.1-1.5 µm in diameter. Conidiophores dark, gray, mostly simple, rather longer, needle shaped, typically bearing simple conidia; 20-30 µm thick, 45 – 80 mm long.

9) Botrytis cinereaPers. ex Pers.

Disease sample: - Fruit

Name of disease: - Botrytis Fruit Rot (Grey Mold Rot)

Causal agent: - Botrytis cinerea

Symptoms of Disease: - Initial infection occurs when fruit are in direct contact with the soil. The fungus also colonizes in dying flowers and fruit through the stem end, growth cracks and wounds. Botrytis also infects cold-injured fruit. Soft rot may develop and consume the fruit entirely. Affected areas are gray to olive green, slightly sunken and have distinct margins. The epidermis peels away easily from lesions to reveal softened, watery underlying tissue. Under humid conditions, gray-brown mycelial develop on the surface, and grape-like clusters of spores can be seen with a hand lens.

Morphology of pathogen: -Colony is white to gray or grayish to brown, and spreading for a short distance in agar. The fungus is characterized by stout, brown, branched conidiophores supporting glistening gray heads of pale conidia. Conidiophores slender, long often darkly colored when viewed through, branching irregularly at end; terminal cells enlarged or rounded and bear clusters of conidia, which can be observed under low magnification of compound microscope. Conidiophores are brown, tall, upright, and septate and branched, up to 25 µm wide and 3 µm long. Conidia occur in clusters at the swollen rounded apices and at intervals along with conidiophores on short blunt teeth. Conidia are oval or egg-shaped, often with a slightly projecting point of attachment, colorless to pale brown, and measure 5-15 x 3 – 4 µm. Large, black, irregular sclerotia produced. They are rather flat in appearance and measure 4x3x3 µm. Conidiophores long, slender, hyaline or pigementd, branched, sometimes dichotomous; conidia hyaline or ash-coloured, gray in mass, 1-celled, ovoid; black irregular sclerotia frequntely produced; parasitic, causing gray mold of many plants.

10) Cercospora personata

Disease sample: - Leaf

Name of disease: - Cercospora Leaf Spot (Frogeye)

Causal agent: - Cercospora personata

Symptoms of Disease: - This disease affects the leaves, petioles, stems and peduncles of chili. Symptoms first appear as small, circular to oblong chlorotic lesions. Lesions later turn necrotic with a sporulating light-gray center and a dark-brown margin. Concentric rings may be observed as individual lesions expand. These lesions

often resemble frog eyes, giving this disease its common name. As the lesions dry, the centers crack and drop out. When the disease is severe, defoliation and reduction in fruit size occur.

Morphology of pathogen: - Conidiophores dark, simple, arising in clusters and bursting out of leaf tissue, bearing conidia successively on new growing tips; conidia hyaline or dark, filform, several celled; parasitic on higher plants, commonly causing leaf spot. Conidia hyaline to lightly pigmented, multi-celled with 5 or more septations, one end may be narrower than other; conidiophores produced in clusters. The fungus produces stromata which are globular. Conidiophores in mass are medium dark and slightly olivaceous brown in colour and paler towards the tip. Conidia are sub-hyaline to pale olivaceous. Conidiophores 20-30 µm in diameter and conidia are long septate 25-55 µm in diameter.

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