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Disease Syndrome and Histopathology of *Protomyces Macrosporous* Galls on *Coriandrum Sativum*

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Abstract

Coriander (*Coriandrum sativum* L.) belonging to family Umbelliferae is an important condiment and occupies a significant place among the non-food crops grown in India. It has a great medicinal value in Ayurveda. Unfortunately, this cash crop of great economic and medicinal value suffers from stem gall disease caused by fungus *Protomyces macrosporous*. This disease has been reported from many parts of India. Present study deals with the study of disease syndrome and histopathology of different parts of diseased Coriander plants.

Keywords Protomyces macrosporous, Coriandrum sativum L, Disease syndrome, Histopathology.

Introduction

India is the largest producer, consumer and exporter of spices in the world and India's spice export is 17,58,985 tons valued Rs. 30973.32 crores during 2020-21. Seed spices have risen as one of the significant groups of spice crop in India.In financial year 2022-2023, the volume of coriander production in India is estimated to have amounted to 847 thousand metric tons. The area under cultivation is 103576 hectare and production is 135810 tons in Rajasthan during 2022-23(Spice board,2023).*Coriandrum sativum* L.is one of the earliest spices known to mankind. It has a great medicinal value in Ayurveda. A strong decoction of the herb given with milk and sugar for the cure of piles (Kirtikar and Basu,1918). The fruit is aromatic, stimulant, carminative, antispasmodic, diuretic, aphrodisiac and refrigerant (Gupta,1962).

The dried fruits are an important ingredient of curry powder and also used in pickling spices, sausages, seasoning, confectionary and for flavouring spirits, particularly gin. The young plants are used in chutney, curries and in soups. Oil of coriander seeds is a valuable ingredient in perfumes (Sambamurty and Subramanian,1989). It is a spices which induces secretion of saliva converting polysaccharide into digestible disaccharides (Agarwal and Sharma,1986).

Coriander is a native of Mediterranean region. In India, it is cultivated in all the states and constitutes an important subsidiary crop. Coriander plant is a smooth, erect, annual herb,30-70 cm high, lower leaves broad with erectly lobed margins, upper leaves finely cut with linear lobes, flowers small white or pink in compound terminal umbels, fruit globular, two seeded, schizocarp, composed of two mericarps. Unfortunately, this cash crop of great economic and medicinal value suffers from many fungal disease viz. stem gall induced by *Protomyces macrosporous*, wilt disease caused by *Fusarium oxysporous*, blight disease caused by *Colletotrichum gloeosporoides* (Srivastav *et al.* 1962).

The stem gall disease caused by fungus *Protomyces macrosporus* on this condiment crop causes severe damage. The disease has been reported in Madhya Pradesh, Uttar Pradesh, Bihar, Punjab and Rajasthan (Gupta and Neergaard,1970).Stem gall of Coriander is one of the most common, widespread and serious disease throughout the world and causes significant losses. (Munish Leharwan and Meenu Gupta,2019). Stem gall caused by *Protomyces macrosporus* is a major disease which causes yield loss up to 33-36% in India. (Sandeep kumar et all, 2018).A preliminary survey in Rajasthan has shown that more than 50% loss in yield occurs due to this disease in Dholpur, Bharatpur, Kota, Jaipur, Alwar, Jodhpur, Swaimadhopur, Bhilwara and adjoining areas. In these areas, mustard has replaced coriander due to stem gall menace. The disease symptoms appear in the form of

small protuberance of stem and as hypertrophied seeds (Gagan kumar and Adesh kumar, 2020). Infection of *P. macrosporus* greatly affect the size and quality of seeds. Infected seeds become excessively larger as compared to normal seeds and the germination capacity of the seeds is also lost. Deformity in size and measurement of infected seeds compared to healthy seeds indicate susceptibility to stem gall disease (Jameel Akhtar *et al*. 2020).

Aim of study

1. To study the disease syndrome of gall disease caused by *Protomyces macrosporous* in coriander.

2. To study the histopathology of galls caused by *Protomyces macrosporous* in different parts of coriander.

Review of Literature

A study was under taken to study the morpho-anatomical, biochemical and physiological changes induced by insect infestation in *Tamarix nillotica.* It was inferred that galled stems showed increased thickness of the periderm and phellem layer and effected the host structure and its cellular metabolism and induced oxidative stress (Younis, A.A.,2021). An experiment was carried out in green house and laboratory condition to know the spread of pathogen and effect on viability of seeds which were collected from different degree of infected plant parts. The lowest seedling infection was observed in apparently healthy seeds (5-10%) in comparison to stem infected seeds (30-40%) (Mishra, R.S. and kumar, S.,2016). The effect of different temperature (15-35%) and moisture levels 15-75 per cent was studied on the development of stem galls of coriander caused by *Protomyces macrosporous* plants grown under controlled conditions. Result revealed that disease development was maximum (77.50%)at 25°C soil temperature followed by 30°C temperature giving 52.50 per cent disease incidence and 30.00 per cent disease severity while very low and high temperature were not favourable for development of disease (Leharwan, M., gupta. M. and Shukla, A., 2018).

Methodology

Survey and Collection

An extensive survey of several fields of Dhania (*Coriander sativum*L.) was carried out in all the 'Dhania' growing areas specially Ajmer, Bharatpur, Bundi, Chittorgarh, Dausa, Dholpur, Jaipur, Jhalawar, Jodhpur, Kota, Swaimadhopur, Sikar and Tonk district of Rajasthan. A general survey of these Dhania growing districts showed that *Coriandrum sativum* was highly susceptible to *Protomyces macrosporus* which causes stem gall disease. In these areas, mustard has replaced Coriander due to stem gall menace. The disease appears in the months of January and March when winter rain occurs and foggy conditions prevail. Normal and *P.macrosporus* infected plant materials of *C. sativum* variety RC-41 were collected from the field of Rajasthan Agriculture college, Jobner campus, Jaipur. Precautions were taken to avoid contamination of the plant material with other microorganisms

A wide range of fungal galls or abnormal growth are known in plants. Fungi causing the galls are known as mycocecidia.The epidemiology, histopathology, biochemistry and control aspects of coriander gall produced by *Protomyces macrosporus* has been studied by many workers (Gupta 1958, Mathur1962, Gupta and Gupta 1962, Mukhopadhyay and Pavgi 1964,1971,1973,1975, Goel*et al.*1983, Maheshwari*et al.*1984, Prasad*et al.*1989, Lakra1990, Sriwastava1992, Paul 1992).

Protomyces is a primitive fungus belongs to Hemiascomycetes. Its mycelium is intercellular and endophytic. The pathogen survives in the soil and forms resting bodies that act as primary source of infection.

Histopathology

Different developmental stages of stem gall and normal counterparts and normal and gall seeds of *C. Sativum* were collected from the fields and bagged in polythene envelopes containing cotton webs, soaked in formic acid. Later on the material was fixed in 70% alcohol or FAA.

For microtomy, the normal and gall material were washed thoroughly to remove all traces of fixing fluids. Subsequently, steps of dehydration, cleaning and embedding were done following tertiary butyl alcohol (TBA) method (Johansen,1940). Microtome section were cut at thickness of 10-12 micron(µ). Section were stained with safranin light green and safranin fast green combination. The safranin light green combination gave best results. DPX mountant was used for mounting. Slides were observed under Nikon Alphaphot trinocular microscope and photomicrographed.

Fungal mycelium of *Protomyces macrosporous* infected host tissue were stained in cotton blue and lacto phenol (Carmichael,1955).

Analysis

Disease Syndrome

Localized and Systemic Infection

Stem gall disease of *Coriandrum sativum* caused by *Protomyces macrosporus* is localized initially but becomes systemic in due course. It is not possible to distinguish an infected plant from a healthy one unless it shows external symptoms. Initially localized symptoms appeared as yellowing speck on the stem of coriander. As the fungus advances in the tissues a bulge arises on the surface of stem in due course. The infected plant exhibits symptoms in the winter season i.e., from early January to late February, but the symptoms may not appear on all parts. Systemic infection was observed 50 to 60 days after the germination of seeds. Galls appeared first on stems, leaves and later at flowering stages as glossy knob like structures. Gupta (1962) and Rao (1972) and Srivastava (1992) have described the disease symptoms.

Diseased Stem Syndrome

Diseased plants were noted from a distance in shady habitat. Infected plants were light (slightly pale) in colour may be due to chlorosis in them (Fig. 1).

The first symptom of the disease was observed on the surface of the stem as a small yellowish speck. This gradually starts increasing in size. As the fungus advances in the tissue internally, a bulge arises on the surface of the stem in due course. The outgrowths are of different sizes and shapes.

The gall is usually elongated, sometimes globular structure. The galls are soft and fleshy when young, generally becoming hard and woody as they grow older. The hardness of the gall is due to the formation of a corky layer outwardly whereby colour of the gall also changes from creamy to light brown (Fig. 2). Usually, the nodal region does not show infection, however in severe cases the entire stem become galled.

Diseased Leaf Syndrome

The infected leaf becomes yellowish in colour. Its mid vein and lateral veins show minute elongated galls. The abnormal growth on the petiole resembles that of the stem in texture and colour but differs from it in size, being smaller. The outgrowth is either seen around the petiole or only on one side (Fig. 3).

Diseased Inflorescence and Flower Syndrome

Peduncles and pedicels of flower showed hypertrophy and became curved downwards due to formation of galls. It is seen in some instances that the flowers and fruits borne on the infected stalks may escape disease. The flowers are also hypertrophied when invaded by the parasite. All the floral parts are susceptible to infection. In severe cases the corolla shrivels, but is not appreciably hypertrophied. All the floral parts of an infected flower may not be invaded by the fungus (Fig. 4).

Diseased Fruit Syndrome

The tissues of the diseased fruit are stimulated to grow by the pathogen, unlike those in a normal fruit. The resulting body becomes bilaterally flattened and enlarged, brittle, straw yellow containing abundant chlamydospores. The diseased fruit is 3-8 times larger than the normal fruit (Fig. 5). Pavgi and Mukhopadhyay (1972) studied in detail development of coriander fruit infected by *Protomyces.*

Histopathology

Structure of Normal Axis

The stem in transverse section showed a well-developed single layered epidermis with stomata (at young stage) and a thin cuticle. The outer cortex is made up of two types of

alternating tissues - chlorenchyma and collenchyma. The inner cortex is 3-6 layered and is made up of isodiametric parenchymatous cells. The vascular bundles are collateral, conjoint and open. Pericycle is 1-2 layered and is composed of thick walled slightly compressed cells. Oil canals were observed in the cortex just below collenchymatous bands. Parenchymatous pith occupies centre of the stem (Fig.6A).

Structure of Hypertrophied (Galled) Axis

The outermost layer of the gall is derived from the epidermis of the stem itself. When the gall is young, the epidermis is almost normal. However, as the gall enlarges, to keep pace with the growing gall, the epidermal cells increase in number by anticlinal divisions of its cells. As a result of infection, the chlorenchymatous cells lose their pigments and ultimately get compressed against the epidermis. The cortical cells and the phloem are first to be invaded by the parasite in the stem. The inner cortex takes a major part in the development of gall. The oil canals lose their characteristic nature and there is intensive mitotic activity in the cortical cells which causes increase in the width of the infected area of the cortex. Hypertrophy and hyperplasia play an important role in the further development of the gall. The mycelium in the cortex differentiates into chlamydospores. The infected cells of the phloem are also larger as compared to the normal phloem. As the galls become older, chlamydospores also develop in the phloem. The xylem is proliferated in a moderately developed gall and is made up of tracheid and xylem parenchyma (Fig. 6B, C).

Structure of Infected and Normal Fruit

The infected fruits are mostly asymmetrical. The parasite invades the septum as well as the ovary wall. The oil canals which are very conspicuous and highly characteristic in a healthy fruit are slightly dislocated from the original position in the galled fruit. The dislocation of the locules may be attributed to asymmetrical growth of the fruit due to infection. A healthy fruit splits up into two mericarps at maturity. On the other hand, the infected fruits do not dehisce or they seem to have lost the capacity to split.

Hypertrophy is usually associated with hyperplasia in the development of diseased fruit. The infected fruits do not develop the thick sclerenchymatous bands which are a normal characteristic of healthy fruit. In a healthy fruit, the vascular bundles are disposed in these bands but in the infected fruits the vascular bundles are embedded in thin walled cells (Fig. 7A, B).

The shape of the oil canals is affected considerably as the invasion of the parasite in the neighbouring tissue gradually increases and they may appear oval to slit like. Sometimes the distance between two canals is markedly changed. In a few instances it was found that even the basic number of the oil canals in a fruit is altered. It is sometimes more in number due to their splitting or become reduced due to their complete obliteration in diseased fruit. If flower buds or young fruits are affected, the ovule aborts and there is no seed development and the space is full of fungal chlamydospores.

Structure of Chlamydospores

The chlamydospores of *P. macrosporus* are yellowish brown, spherical or globose to oval, single celled, smooth and measured 40 to 81 µ in diameter. A mature chlamydospore is protected externally by a hard, thick and unsculptured exospore enclosing a medium thick mesospore and a membranous endospore. The protoplasmic contents are densely granular in appearance (Fig. 8).

Coriander plants infected severely with stem gall disease lose their vigour and are deformed in their vegetative and reproductive growth forms. When inflorescence is attacked seed production is drastically reduced causing significant loss in yield.

Result and Discussion

Stem gall of *Coriander sativum* caused by fungus *Protomyces macrosporus* during month of January to march. *Protomyces* generally attack stem, inflorescence and fruit of *Coriandrum sativum* making hypertrophoid galls.

In general, both hypertrophy and hyperplasia of cells take place completely changing the normal arrangement of cells in the host tissue (Akai,1951). Generally, the epidermal cells of fungal galls increase in number which occurs during the gall development. The divisions are normally anticlinal and keep pace with the developing galls. In this case also the epidermal cells are found stretched eventually and become narrow in diameter.

The principal process that are responsible for production of galls in the host plant are the same in all cases. However, tissue involved is different in different host plants depending on the parasite action. In the present study, in case of stem galls, the parasite invades the cortical cells and stimulate them to hypertrophy,sometime followed by hyperplasia. When the parasite reaches the phloem tissue the cells are activated to cell division. The tissues thus produced results in outgrowth on the surface of the host organs involved.

The fungal mycelium in the cortex and phloem differentiate into chlamydospores. Similar observation was also made by Rao (1972) and Srivastava (1992). Galled fruits of *Coriandrum sativum* is 3-8 times larger than normal fruit and it is full of fungal chlamydospores. The infected fruit do not develop thick sclerenchymatous bands, which are a characteristic of normal fruit.

Fungal galls may show in many cases elaborate vascular system or a simple vascular supply like that of normal host organ. In the younger parts of the hypertrophied stem of *Crepis japonica* attached by *Protomyces inouyci* (Akai,1939) and also *Protomyces* galls on *Ambrosia nifiela* (Stewart,1916) reported formation of numerous parenchyma new strands.Accompanied by short tracheid these strands some time develops into vascular bundle(Akai,1939).

In majority of fungal galls pith is not affected by the parasite. In general, whenever it is infected, the parasite is found to enter through the medullary ray region. However, the pith in this case is invaded by the parasite through the unlignified interfasicular region. When the invasion by the parasite is severe, the pith cells are found very much crumbled.

The effect of different biocontrol agents and fungicides on crop yield for stem gall disease of coriander caused by *Protomyces macrosporous* unger. The effectiveness of plant leaf extracts, fungicides and bio agents were evaluated against the test fungi under in vitro conditions and further evaluated under field conditions for disease management and crop yield management (Vijay and kaushal, 2021)



FIG. 1