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Defense Related Anatomy in Infected Indian Mustard Plant in the Field

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ABSTRACT

Disease and insect infestation and defense related anatomical study was attempt in Indian mustard [*Brassica juncea* (L.) Czern & Coss] as a newly introduced variety at the Barind tract of Bangladesh. An experiment was conducted at field and laboratory of EXIM Bank Agricultural University Bangladesh, Chapainawabganj. Disease and insect infestation rate were measured in the collected samples from the field cultivated. Defense related mechanisms in infected plants examined with anatomical and histohemical studies after sectioning infected samples of Indian mastered. Structural defense related changes observed anatomical while histochemical changes defined phenolic deposition the cells and cell walls with Fast BB salt stained. As major infection, Alternaria leaf blight and Cercospora leaf spot was detected. Alternaria leaf blight infection rate (86.1%) was higher than the Cercospora leaf spot (13.9%). Insect infestation especially aphid infestation was considerable in Indian mustard. Defense mechanisms such as hypersensitive response, abscission layer formation, cell wall thickening and phenolic compound deposition in the tissues were found in infected leaves.

Keywords: Alternaria leaf blight, Cercospora leaf spot, Defense mechanism, Diseases, Indian mustard, Insects

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1. INTRODUCTION

Many factors are associated with the poor yield of mustard-rapeseed in Bangladesh. Diseases have been identified as one of the major factors (Ahmed, 1992: Hossain et al., 2018). Brassica oilseeds are suffered from thirty diseases (Khan, 2011). Common diseases of mustard-rapeseed are Aternaria leaf spot, Stem rot, Stem canker, Club root, White rust, Light leaf spot, Downy mildew, Turnip Mosaic Virus (TuMV) and Turnip Yellows Virus (TuYV) (Snowdon et al., 2007). On the other hand, Alternaria blight is the most serious and devastating disease of mustard in Bangladesh (Ahmed & Ahmed, 1994). The disease causes blight of leaf, pod and stem and seed abnormalities (Howlider et al., 1991). It is endemic in the country and most of the cultivated varieties are susceptible to the disease. The disease causes yield losses of 40-70% in India (Vishwanath & Kolte, 1997) and 30-60% in Bangladesh (Ahmed & Ahmed, 1994; Meah & Hossain, 1988). In addition to direct yield losses, the disease adversely affects the seed quality by reducing seed size and causing seed discoloration and reduction in oil contents (Howlider et al., 1991). Pod of mustard is the main component of seed yield and normal filling of seed takes place if pod can be protected from infection (Hossain & Mian, 2004).

The attributions of the hosts that reduce the chances of infection or the further development of the pathogen are considered to be defense mechanism (Gowthami, 2018). In

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general, plants defend themselves against pathogens by a combination of weapons from two arsenals: (1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and (2) biochemical reactions that take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant (Agrios, 2005).

After the establishment of infection in plant cells, the host defense system tries to create barriers for further colonization of tissues is called histological defense mechanisms. Those are formation of cork layers, formation of abscission layers, deposition of gums, formation of tyloses and necrotic structural defense reaction (Defense through the Hypersensitive Response).

Mustard aphid, *Lipaphis erysimi* (Kalt.); mustard sawfly, *Athalia lugensproxima* (Klug.); painted bug, *Bagrada hilaris* (Kirk.) and the leaf miner, *Phytomyza horticola* (Goureau) are the major pests of mustard. The mustard aphid, *L. erysimi* is the key pest in all the mustard growing regions. Nymphs and adults of the aphid suck cell sap from the leaves, inflorescences and immature pods resulting in very poor pod setting and yield. On the other hand, aphid produces a good amount of honeydew which facilitates the growth of sooty mould that makes the leaves and pods appear dirty black, which ultimately hampers the process of photosynthesis (Dotasara et al., 2018). In terms of economic importance, mustard aphid, *L. erysimi* is regarded as a national pest (Rao *et al.*, 2014), considered to be a major limiting factor in successful cultivation of the crop; reducing yield to the tune of 35.4-96.0% and oil content of 5-15 per cent (Bakhetia & Sekhon, 1989; Singh & Premchand, 1995).

Indian mustard (*Brassica juncea* L. Czern & Coss) is a HYV oilseed crop belonging to family Brassicacae. Indian mustard was originally introduced from China into northern India from where it has extended to Afghanistan via Punjab (Vaughan, 1997; Sambamurthy & Subramanyam, 2000). This species originated from the hybridization of *Brassica nigra* with *Brassica campestris* and this probably happened in southwestern Asia and India where the natural distribution of the two species overlaps (Saucer, 1993). Seeds are small and contain 38-42% oil (Shekhawat et al., 2012). It yielded 2300 - 2400 kg/ha (Junjariya, 2014).

As a promising oil seed crop, the research work on Indian mustard is very limited without productivity test in the field. Ghosh & Chatterjee (1988) conducted a research on Rabi season. They tried to determine the effect of sowing date on the productivity of Indian mustard in their research area. Woods et al. (1991) conducted a study on Brassica juncea (L.) Cossin Western Canada about the potentiality of that variety.

Mustard is the most important oil seed crop in Bangladesh. There are huge shortages in the production of edible oil in Bangladesh. For meeting the demand of edible oil to consumers, it is needed to import a huge amount of vegetable oil. To increase the yield of mustard we introduced a new HYV mustard (*Brassica juncea* L. Czern & Coss) at Barind tract, Chapainawabganj district in Bangladesh. As the part of introduction of this mustard, it is important to examine the disease and insect infestation rate and susceptibility to disease and insect attacked. Therefore, the present study was attempted to investigate disease and insect infestation rate and defense related mechanism in the anatomical structure in infected Indian mustard plant.

2. MATERIALS AND METHODS

Indian mustard (*Brassica juncea* L. Czern&Coss), a tall variety of mustard was cultivated at the main campus of EXIM Bank Agricultural University Bangladesh located at Jhilim 3, Amnura, Chapainawabganj district under Agroecological Zone (AEZ) of 10 (Active Ganges Floodplain), 11 (High Ganges River Floodplain)and 26 (High Barind Tract).

The experiment land was 1.8 bigha. The land was prepared by cross ploughing. The fertilizers were applied according to the recommended dose of AEZ 10, 11, and 26. Urea (55 kg/1.8 bigha), TSP (39.6 kg/1.8 bigha), MoP (36 kg/1.8 bigha), Gypsum (21.6 kg/1.8 bigha), $ZnSO_4$ (2.2 kg/1.8 bigha) and Boric Acid (1.8 kg/1.8 bigha). Seeds were sown (1 kg/bigha) by broadcasting method. After emergence of seeds, weeding and thinning were done once at 25 DAS. Urea was applied two times. First half at the time of land preparation and the rest part were top dressed. Top dressed was done with half amount of urea at 55 DAS and half was used before at the time of ploughing. Two irrigations were applied: first irrigation was given at 7 DAS (days after showing) and second irrigation was given at flowering stage (75 DAS).

Diseased plants were collected from the experimented field and observed the spots for identifying the disease infection. By counting the spots over the leaves surface, leaf spots were classified and disease infestation rate were calculated. The conidia of the pathogen were isolated from infected leaf. For identification of pathogen, glycerin-added brush wiped on the diseased affected surface and then trampled on the slide. After mounting cover slip, sample was observed under light microscope. Aphid were collected from the field and identified under Stereo microscope.

The anatomical changes caused by pathogenic attack were examined in the transverse sections of infected leaf. For the preparation of transverse section of leaf, thin and uniform transvers sections were cut with razor blade by hand. The sections of leaves were stained with Fast Blue BB Salt for detection of phenolic compounds. Fast Blue BB Salt has the ability to react specifically with OH- group of phenolic compounds (Soukupova et al. 2000) to give a characteristic reddish-brown reaction product. A compare anatomical study was conducted within non-infected healthy and infected leaves.

3. RESULTS

3.1. Disease Infection Rate in the Field

Healthy leaf and inflorescence of Indian mustard were shown in Fig. 1A. The leaves with ovate or obovate shape

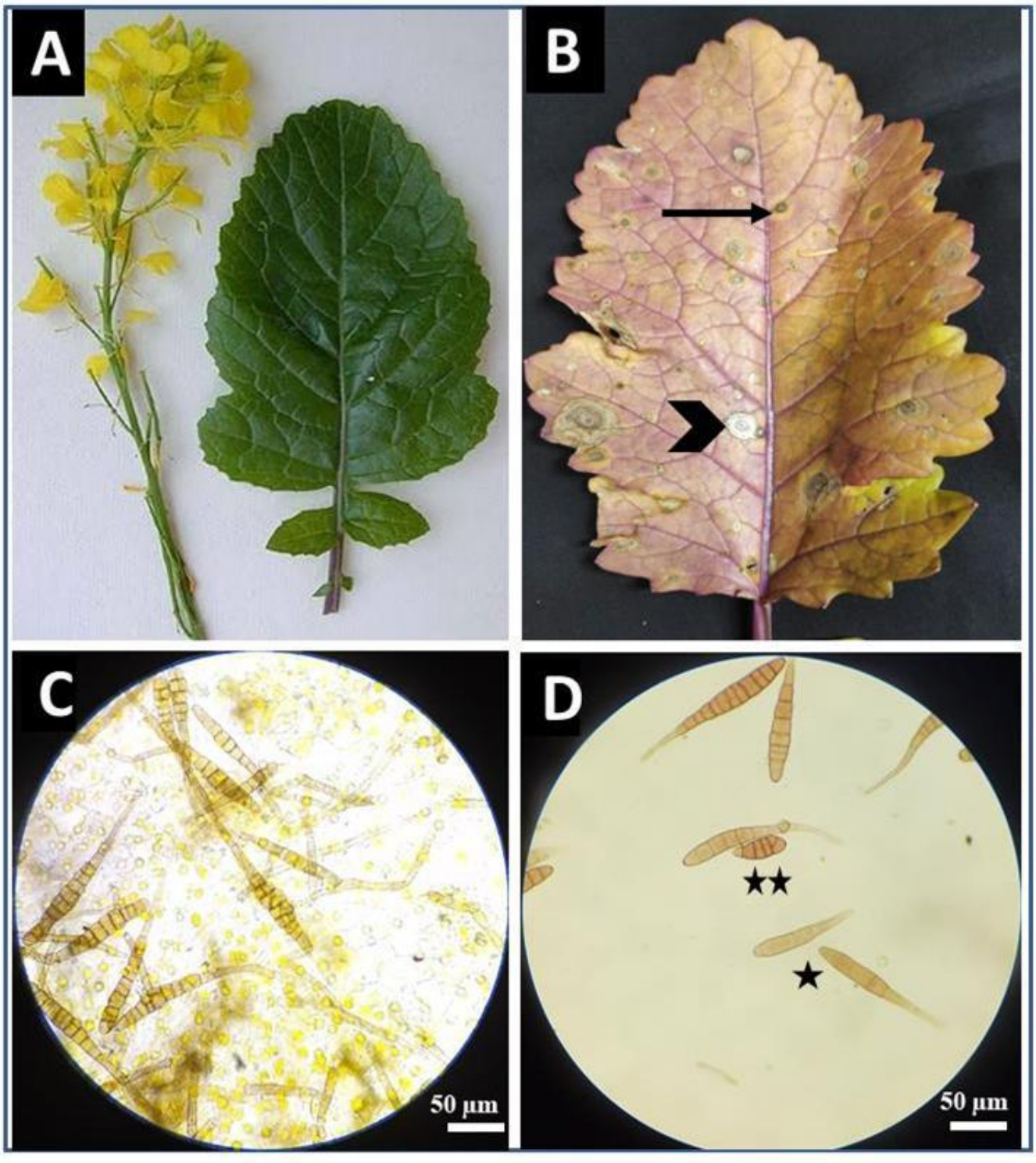

Fig. 1 Disease in the infected leaf of Indian mustard. A) Healthy leaf and inflorescence. B) Diseased leaf Alternaria leaf blight and Cercospora leaf spot. C. Conidia and conidiophore on infected leaf. D) Isolated conidia of *Aternaria* spp., *Alternaria brassicae* and *Alternaria brassicicola.* Arrow (Cercospora leaf spot), Arrowhead (Alternaria leaf blight), Single asterisk (*Alternaria bassicae*) and double asterisk (*Alternaria brassicicola*).

are simple and petioled; the flowers of the raceme inflorescences were bisexual with four free sepals and four yellow petals, along with two longer and two shorter stamens.

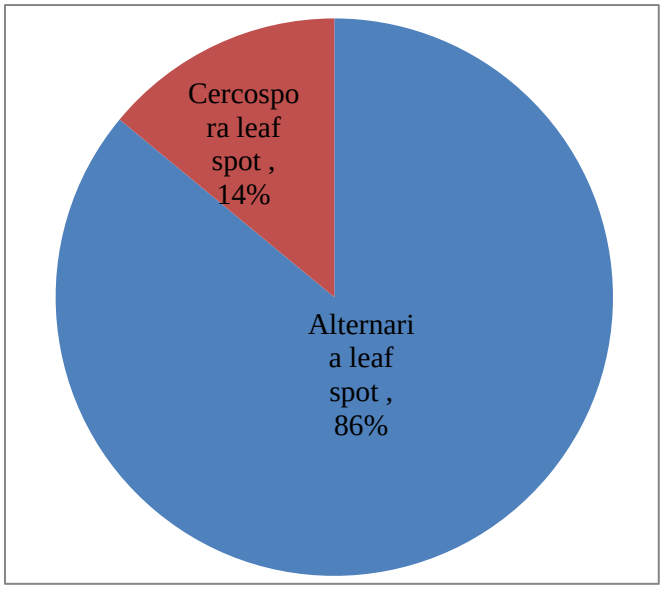

Fig. 2 Disease infection rate in field of Indian mustard.

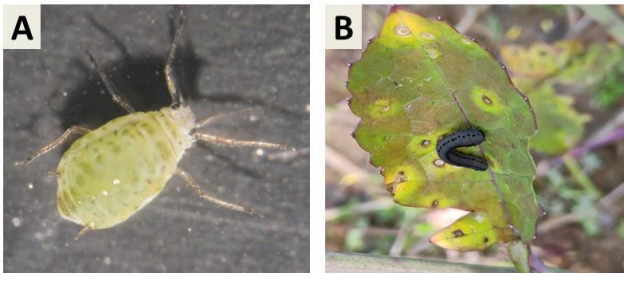

Fig. 3 Pest of mustard. A. Mustard aphid B. Mustard caterpillar.

Indian mustard was very susceptible to leaf spot disease that was examined in the research field too. Two types of leaf spot disease were found in Indian mustard in the present study, i) Alternaria leaf spot disease and (2) Cercospora leaf spot disease. The fungus *Alternaria brassicae* and *Alternaria brassicicola* was causal agent for the Alternaria leaf spot disease and Cercospora leaf spot disease, respectively. The rate of Alternaria leaf spot and Cercospora leaf spot disease were 86% and 14%, respectively (Fig. 2).

Alternaria leaf spot disease had concentric rings of raised and depressed tissue as new lesion develop and older lesion expand the entire leaf becomes chlorotic (yellow) (Fig. 1B). Alternaria leaf spot disease squeezed as severe condition on the other hand Alternaria leaf spot disease sprayed very small area that was ignorable. Conidia and conidiophore in diseased leaf were showed (Fig. 1C) caused by *Alternaria brassicae*. The conidia of *Alternaria brassicae* have long beak called *Alternaria brassicae*.

Cercospora leaf spot disease was small, round, yellow and slightly sunken appeared on the upper leaves. As spot grows, it had brown center surrounded by purple color margin and those were the characteristic features of this leaf spot disease (Fig. 1B). The conidia are dark brown and smooth-walled. The conidia are cylindrical to oblong in shape and are muriform and produced in chains of 8-10 spores. *Alternaria brassicicola* were found in the collected samples the conidia that have short beak called *Alternaria brassicicola* (Fig. 1D).

3.2. Insect Infestation Rate in the Field

Insect infestation was considerable in Indian mustard. When temperature increases, attack of aphid and mustard caterpillar were noticed. Aphid infestation (Fig. 3A) was higher than mustard caterpillar (Fig. 3B). Mustard caterpillar infestation was ignorable.

3.3. Defense Mechanisms through Anatomy

There were four types of defense related mechanism perceived in Indian mustard leaves in the present study. Throughout the cytoplasm, additional brown color with Fast Blue BB Salt was observed in infected section in compared to non-infected section (Fig. 4D). It is clarified that the availability of accompanying phenolic compounds was deposited at the cytoplasm of the cells of infected side. On the other hand, a resin like granules was also observed in their (Fig. 4A). In the sections of infected leaves, radish to brown colored cell wall found at the infected portion confirmed that phenolic compound deposition occurred in the cell wall for cell wall thickening to defense plants from fungal attack (Fig. 4B).The dark brown to black staining in the cell with Fast Blue BB Salt observed at infected area in transverse sections of infected leaves. It was indicated the hypersensitive response of the tissues that was a necrotic structural defense reaction (Fig. 4A).

In the section of infected leaf, cells of tissues phenolic compounds deposition were observed. Phenolic compound also serves as a defense in plant cells. The reddish compound in the cells is recognized the phenolic deposition (Fig. 4B).On the other hand, the changes were not observed in the non-infected leaf (Fig. 4D).

Abscission layer was found on the infected leaf. The discarding infected area from the infected portion is showing formation of abscission layer in leaves of Indian mustard (Fig. 4C).

4. DISCUSSION

In the present study, Indian mustard shows four types of histological defense to protect from pathogens. Indian mustard shows necrotic structural defense reaction (defense through the hypersensitive response). In many host–pathogen combinations, as soon as the pathogen establishes

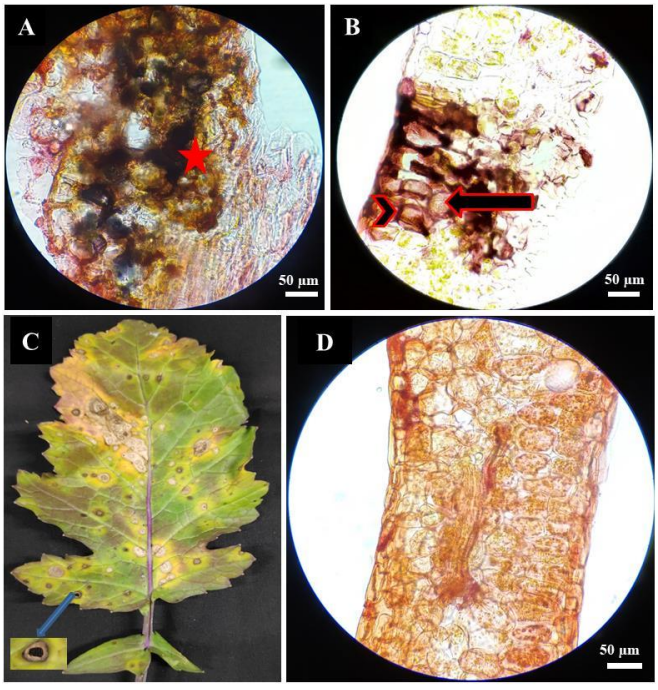

Fig. 4 Defense mechanisms in infected leaves of Indian mustard. A) Necrotic structural defense reaction (Defense through the hypersensitive response). B) Cell wall thickening and phenolic compound deposition. C) Formation of abscission layers on the infected leaf. Single asterisk indicates hypersensitive response, Arrow (Phenolic compound deposition), Arrowhead (Cell wall thickening). D) Transverse section of non-infected leaf.

contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates. At the same time, brown, resin-like granules form in the cytoplasm, first around the point of penetration of the pathogen and then throughout the cytoplasm. As the dark browning discoloration of the plant cell cytoplasm continues and death sets in, the invading hypha begins to degenerate. In most cases the hypha does not grow out of such cells, and further invasion is stopped (Agrios, 2005).

By the attack of pathogen Indian mustard produce abscission layer. Abscission layers are formed on young, active leaves, an abscission layer consists of a gap formed between two circular layers of leaf cells surrounding the locus of infection (Agrios, 2005).

The plant cell wall is a complex network composed of various polysaccharides. The presence of cellulose, hemicellulose and pectin is a common feature for all plants' cell wall (Cosgrove, 2005). Alterations in the composition or structure of the cell wall have been demonstrated to affect plant resistance against biotic stresses. In most cases, those mutants are increased in disease resistance, indicating the trade-offs between growth and disease resistance (Schulze et al., 2010; Ramirez et al., 2011).

Xylan and xyloglucan both belong to hemicellulose and are also main components of the plant cell wall. Growing evidence demonstrates that changes in xylan or xyloglucan affect disease resistance of Arabidopsis to pathogens (Delgado-Cerezo et al., 2012; Sampedro et al., 2010; Chowdhury et al., 2017). Hemicellulose acetylation affects biotic invasion by determining the cross-link of polysaccharides in the cell wall (Gille & Pauly, 2012).

After pathogens break the outmost cutin layer of plant epicuticle, pectin serves as the first barrier to prevent invasion. Owing to this fact, alterations in pectin component or modification affect plant disease response. Pectin is generally secreted from the golgi apparatus to the cell wall in a highly methylated form. Pectin methylesterases (PMEs) remove the methyl group of homogalacturonan during incorporation of pectin into the cell wall (Harholt et al., 2010; Sterling et al., 2001). It has been suggested that highly-methylated pectin shows well tolerance to the attack by cell-wall degrading enzymes (CWDEs) secreted by pathogens, thus conferring plant with disease resistance (Raiola et al., 2011).

Lignin, a hydrophobic aromatic polymer, is usually present on the secondary cell wall of vascular plants (Chen & Dixon, 2007). Lignin content is typically positively correlated with plant disease resistance. Higher lignification of the cell wall has been observed in plants exposed to pathogen infection or deficient in cellulose biosynthesis, thereby increasing mechanical strength of the plant cell wall and improving tolerance of the cell wall towards CWDEs released by pathogens (Hernandez-Blanco et al., 2007; Huckelhoven, 2007).

In the sectioned leaves, the cell wall were thicker than the normal cell wall that showing defense in Indian mustard by thickening the cell wall.

Phenolic compounds regulate crucial physiological functions in plants to provide resistance against various biotic and abiotic stress conditions. Plants accumulate phenolic compounds at infections site to reduce growth and penetration of microbial pathogens in other tissues and organs. It recognizes microbial pathogens and induces defense response at genetic level to biosynthesize defense metabolites. Plants also accumulate salicylic acid and H_2O_2 at infection site to regulate systemic acquired resistance. Plants accumulate phenolic compounds in organs which acts as inhibitor or toxicants for nematodes, insects and herbivores (Chowdhary et al., 2021).

In the present study, the attack of mustard aphid was higher than mustard caterpillar. Singh et al. (2010) reported that the mustard aphid, *L. erysimi* damaged the crop from vegetative stage to maturity, the highest population occurred during the flowering and siliquae formation inflicting a reduction in seed yield of 90.0-93.2% as compared with insecticide treated plots. Tomar (2017) also reported that the avoidable yield loss due to mustard aphid, *L. erysimi* as 88.72 and 90.52% during two consecutive years of study. Loss in yield of mustard crop due to infestation of mustard aphid, *L. erysimi* was estimated by from two sets of ten healthy plants grown in caged area. The yield loss was 84.96, 89.53 and 74.57%, respectively in 2002-03, 2003-04 and 2004-05, which corroborate the present findings.

5. CONCLUSION

Indian mustard has a very poor growth and yield performance in Barind tract. Indian mustard was very susceptible to Alternaria leaf blight disease. Alternaria leaf blight infestation rate was higher than the Cercospora leaf spot rate. Indian mustard showed different types of defense mechanism. It had hypersensitive response (HR), abscission layer formation, cell wall thickening and induce defense related phenolic compound. Aphid attack was found but it was not so severe. This research was a new initiative for this perspective indeed.

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