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# Artificial and Natural Management of Common Infectious Bioagents of Mustard

**Anubhuti Sharma[1*], Meghna Garg[2] and P. K. Rai[1]**

[1]ICAR – Directorate of Rapeseed- Mustard Research, Sewar, Bharatpur, Rajasthan, India

[2] Department of Biotechnology, GLA University, Mathura, Uttar Pradesh, India

Corresponding author*: sharmaanubhuti98@gmail.com

## ABSTRACT

Mustard is one of the most important oilseed crops of India. Pathogenic and pest attacks due to *Alternaria brassicae, Albugo candida, Perenospora parasitica,* and aphids, bugs *etc.* can compromise production & quality. Though humans have managed to suppress these pathogens with the use of chemical pesticides & fungicides, the nature has its own way to deal with these attacks. Mustard plants host a diverse range of phytoalexins which work as antibiotics for a broad spectrum of bacteria, fungi and insect pests. Brassilexin, cyclobrassinin, cyclobrassinin sulfoxide, indole-3-acetonitrile, spirobrassinin are some of the phytoalexins identified in different varieties of mustard. This article identifies the various ways of managing these pathogens with special reference to key molecules, phytoalexins with their mode of functioning.

## INTRODUCTION

The family Brassicaceae (syn. Cruciferae) comprises of economically important crops, including Rapeseed Mustard, vegetable and fodder crops. Oilseed crucifers (mainly *Brassica* sp.) constitute the second largest source of edible vegetable oils in India. However, infestations by the pathogens can lead to several diseases like alternaria blight, white rust, downy mildew etc. which damages the crop (Shin et al., 2014; Walton 1997; War et al., 2018).

**Important diseases**

1. **Alternaria blight**

   **Causative agent:** *Alternaria brassicae* and *Alternaria brassicola*

   **Symptoms:** The disease is characterized by the formation of prominent, light brown to black round spots with concentric rings of various sizes on leaves after 40-45 days of sowing that later spread on stem, its branches and silique leading to subsequently blighting and defoliation. (Fig.1)

**Favourable conditions:** Moist (more than 70% relative humidity), warm weather (12-25 °C) & intermittent rains favours disease development. The pathogen survives through spores (conidia) or mycelium in diseased plant debris or on weeds.

**Management:** The plants are sprayed with Iprodione or Mancozeb (Dihane M-45) at the concentration of 2g/litre of water, normally at 50-70 days after sowing.

2. **White rust**

**Pathogen:** *Albugo candida*

**Symptoms:** White creamy raised pustules (Fig. 2a) occur onlower surface of leaves which later turn to patches.However, in mixed infection of white rust and downy mildew, hypertrophy & hyperplasia causes swelling and distortion of the stem and floral parts& a "stag head" (Fig. 2b) structure develops.

**Favourable conditions:** Moist (more than 90% relative humidity) coupled with intermittent rains favours disease development.

**Management:** The crops are sprayed with Ridomil MZ 72WP or Mancozeb (Dithane M-45) with concentration of 2g/litre of water soon after the disease appears. The spray is done at the intervals of 15-20 days.

**Fig.1 Yellow-brown patches on leaves**

**Fig. 2(a) White postules on leaves**

**Fig. 2(b) Stag Head structure**

3. **Downy mildew**

**Pathogen:** *Peronospora parasitica*

**Symptoms:** White fluffy growth of the fungus appear on the lower surface of the leaves occurs due to sporulation (Fig. 3) which slowly spread to stems and stag head formation occurs. Old lesions become necrotic and translucent after invasion by secondary saprophytes.

Seedlings may be killed or develop dark brown vascular systems due to severe infections, but older plants are rarely killed.

**Favourable conditions:** Atmospheric temperature in the range of 10-20 ℃ and relative humidity>90% favours disease development.

**Management:** The crops are sprayed with Ridomil MZ 72WP or Mancozeb (Dithane M-45) with concentration of 2g/litre of water. Pesticide Azoxystrobin (Quadris) can also be used.

4. **Sclerotinia Stem Rot**

**Pathogen:** *Sclerotinia sclerotiorum*

**Symptoms:** Elongated water soaked lesions (Fig. 4) appear on stem near to the crown region, covered with cottony mycelial growth.Later onplant looks like whitish from distance at internodes or base. Premature ripening and shredding of stem, wilting and drying occurs. Brown to black sclerotial bodies may also be seen at later stage on the infected plant parts.

**Favourable conditions:** High humidity (90-95%) and average temperature (18-25 $^{o}$C) along with wind current favours the disease development.

**Management:** The plants are sprayed with Carbendazim (Bavistin) with concentration of 2g/litre of water at 60-65 days of maturity.

**Fig. 3 White fluffy patches on underside of leaves**

**Fig. 4 White lesions on stem**

**Important insects**

1. **Mustard aphids**

Mustard aphid (both nymph and adult) feeds on different parts of the plants (inflorescence, leaf, stem, twig and pods) by sucking the cell sap and heavy infestation can dry up the whole plant. Low temperature and high RH favors the rapid multiplication of aphids.

**Management:** The infested twigs are plucked and destroyed at initial attack of aphids. The plants are sprayed with Dimethoate 30EC at the concentration of 1ml/litre of water. Neem based environment friendly insecticides, syrphid and lacewing, etc. can also be used to minimize the incidence.

**Fig.5 Mustard aphids**

**Fig. 6 Painted bug**

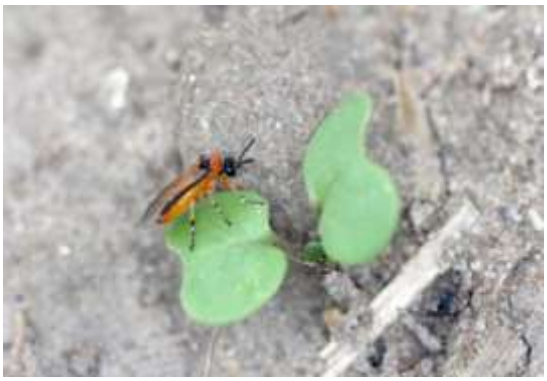

**Fig. 7 Mustard sawfly**

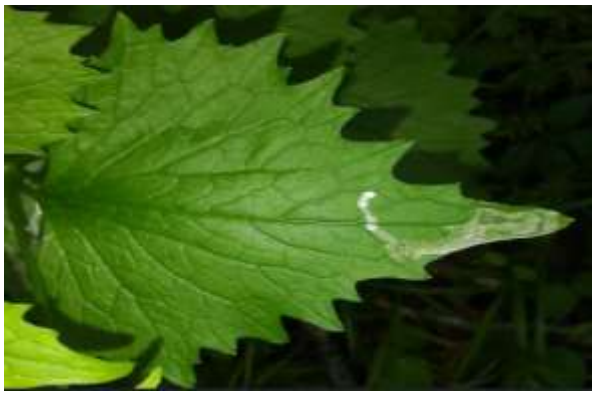

**Fig. 8 Leaf miner**

2. **Painted bug**

Painted bug is a polyphagous pest. Painted bug attacks the crop in warmer months under low moisture condition. The vegetative stage results in whitening of leaves & then wilting, leading to complete drying of the tender shoot/plant.

**Management:** Dust the crop with Quinalphos1.5% © 20-25kg/ha. Spray the crop with Malathion 50 EC ©500 ml in 500 litres of water/ha in case of severe infestation during early stages.

3. **Mustard sawfly**

The pest makes irregular holes in the leaves and in severe infestation the crop looks as grazed by animals.

**Management:** Timely irrigation prevents the attack of the pest. Dust the crop with Quinalphos 1.5% © 20-25kg/ha.

4. **Leaf miner**

The pea leaf miner attacks the crop from sowing to maturity. The maggots of the insect mine the leaf and a large number of silvery zig-zag mines appear due to the feeding on the parenchyma tissues.

**Management:** Foliar spray of Dimethoate 30 EC ©1ml/litre of water.

**Phytoalexins: Plant's defense mechanism against infectious bioagents**

Phytoalexins comprise a large number of low molecular weight metabolites with diverse structures such as isoprenoids, flavonoids, and stilbenes, many of which act as broad spectrum antibiotics against pathogenic fungi and bacteria (Agrios 2005). They are produced only in response to a pathogenic attack and remain undetectable in uninfected plant tissues. In response to an elicitor, which may be constituents of the fungal cell wall, such as glucans, chitosan, glycoproteins, and polysaccharides from the attacking pathogen they accumulate at the sites of infection in concentrations which are inhibitory to the development of fungal and bacterial cells (Harborne 1993; Umezawa & Shin 1999)). When phytoalexin synthesis is stimulated with elicitors, *de novo* transcription of the genes encoding phytoalexin biosynthetic enzymes starts within minutes. Once synthesized, they act as multisite toxicants i.e, capable of affecting a variety of physiological and biochemical pathways. Phytoalexins with isoflavonoid structures can cause membrane dysfunction. Moreover, the lipophilicity of many phytoalexins suggests that their site of action may be the cell membrane. It has also been proposed that isoflavonoid derivatives uncouple oxidative phosphorylation in the fungal cell, leading to cell death (Shibamoto et al., 1993).

Phytoalexins may either be products of the shikimic acid (phenyl propanoid) pathway from which many other plant secondary metabolites (particularly flavonoids), lignin and anthocyanins are also derived or mevalonic acid pathway e.g., gossypol from cotton, casbene from castor bean, and rishitin from solanaceous plants. Till date, more than 300 phytoalexins from 30 different families have been isolated in plants.

Phytoalexins can accumulate in plants or cell cultures only transiently, because they are degraded oxidatively or polymerized by enzymes, such as extracellular peroxidases. Sometimes, pathogens use this property to degrade the phytoalexins & evade the host system e.g., phytoalexins of

the cruciferae family such as brassinin are detoxified to indole-3-carboxaldehyde using glucosyltransferase in *Sclerotinia sclerotiorum*. Similarly, Brassilexin is also detoxified to glucosyl or non-glucosyl derivatives by *S. sclerotiorum*. *S. sclerotiorum* is also able to transform camalexin into the glycosylated derivate at N-1 or C-6 of the indole ring (Soledade et al., 2007).

Some of the phytoalexins present in mustard along with their elicitors are listed below (Table 1). Also, phytoalexins and their biological activity against pathogen has also been listed separately (Table 2).

**Table 1: Phytoalexins present in some common varieties of mustard & their elicitors.**

| **Species (common name)** | **Elicitors** | **Phytoalexins** |
|---|---|---|
| *B. juncea* (brown mustard) | $CuCl_2$, $AgNO_3$; *Alternariabrassicae* | Brassilexin, cyclobrassinin, cyclobrassinin sulfoxide, indole-3-acetonitrile, spirobrassinin |
| *B. napus* (rapeseed) | $CuCl_2$ | Brassilexin, cyclobrassinin, cyclobrassinin sulfoxide, 1-methoxybrassinin, spirobrassinin |
| *B. rapa* (rapeseed) | $CuCl_2$ | Brassilexin, cyclobrassinin, cyclobrassinin sulfoxide, 1-methoxybrassinin |
| *B. rapa* (canola) | *Albugo candida* | Rapalexin A, rapalexin B |
| *Sinapis alba* (white mustard) | Destruxin B, $CuCl_2$, *A. brassicae* | Sinalbin A, sinalbin B, sinalexin |
| *Sinapis arvensis* (white mustard) | *A. brassica* | Brassilexin, cyclobrassinin sulfoxide |

**Table 2: Biological activity of the phytoalexins present in mustard**

| **Phytoalexins** | **Biological activity** |
|---|---|
| Brassilexin | Antimicrobial against *Alternariabrassicae*&*S. sclerotiorum*(cytotoxic effects) |
| Cyclobrassinin | Antimicrobial against *A. brassicae*, *S. sclerotiorum*, *P. parasiticia*. (cytotoxic) |
| Cyclobrassinin sulfoxide | Antimicrobial against *C. cucumerinum* (*C. cucurmerinum* causes fungal disease in mustard) |

| | |
|---|---|
| Indole-3-acetonitrile | Antimicrobial against *S. sclerotiorum, A. brassicae* |
| Spirobrassinin | Antimicrobial against *P. parasitica* & *S. sclerotiorum* |
| Methoxybrassinin | Antimicrobial against *S. sclerotiorum, A. brassicae, C. cucumerinum* (antiproliferative activity) |
| Rapalexin A | Antimicrobial against *Albugo candida* |
| Rapalexin B | Antimicrobial against *A. candida* |
| Sinalbin A | Antimicrobial against *L. maculans* (fungal disease) & *A. brassicae* |
| Sinalbin B | Antimicrobial against *L. maculans* |
| Sinalexin | Antimicrobial against *C. cucumerinum, A. brassicae* & *S. sclerotiorum* |

**Defense mechanism of mustard against insect pest**

Mustard plants are attacked by a large number of insect with aphid being the most devastating insect on oilseed crops. It leads to an average loss of about 50% seed yield. Mustard plants have adapted number of defense strategies against these pests.Plants resist pathogen attacks by activating wide variety of mechanisms, either constitutive or inducible, which contribute to the resistance of plants to pathogens. Constitutive defenses are pre-existing resistance mechanisms which control infection immediately after exposure of host to pathogen. The constitutive defenses include preformed physical (the cuticle, cell wall, waxy epidermal cuticle, stomatal aperture or lenticel) as well as chemical barriers (including inhibitory compounds or the absence of stimulatory compounds needed for pathogen development), which not only protect the plant from invasion of pathogen but also give strength and rigidity to the plant. The inducible defenses are associated with rapid and effective activation of cellular defense responses, which are induced only after contact with a (challenging) pathogen. The induced resistance is activated not only at the site of the initial treatment but also in distal, untreated plant parts.

The establishment of battling response by the plant against a pathogen is correlated with a number of phenomena.

- Firstly, there is a change in membrane permeability by loss of cellular electrolytes such as K+ and an uptake of H+ along with an influx of Ca2+.
- Second, rapid transcriptional activation is seen for a large number of genes around the infectious site. The physical and biochemical changes include lignification and suberization of

the plant cell wall, de novo synthesis of pathogenesis related proteins (PRP's), biosynthesis and accumulation of secondary metabolites and phenolics, which act as antimicrobial compounds, termed as phytoalexins.

- This is followed by rapid oxidative burst which generates high levels of reactive oxygen species that initiate membrane lipid peroxidation and cell death.
- Third, localized necrosis and cell death occurs at the infection site termed as hypersensitive response (HR). All these events together constitute 'localized acquired response' (LAR). LAR is a rapid response which is expressed locally at the site of infection; as a result, the pathogen remains confined to that necrotic lesions near the site of infection.
- Systemic signaling through the plant phloem to secondary tissue results in gene activation in those tissue and triggers onset of an often long-lived broad spectrum 'systemic acquired resistance' (SAR). This activated SAR plays a crucial role in defense of plant by providing durable protection against challenge infection by a broad range of pathogens.

The key for an effective activation of a defense response in the plant is a rapid detection of molecule referred as "elicitors". Elicitors are defined as pathogen/host metabolite that helps in activating the defense response in plants. Elicitors may be classified as exogenous and endogenous. The former arises from hydrolysis of pathogen components by enzymes and the latter from hydrolysis of plant components by plant enzymes (Boller, 1995).

The ability of many plant pathogens to cause disease on a particular host is regulated by gene-for-gene interactions between resistance (R) genes in the host and specific avirulence (Avr) genes in the pathogen (Flor, 1956). The recognition of an Avr gene product by the corresponding R gene product elicits plant defense mechanisms that produce an incompatible interaction (Nimchuk et al., 2003); whereas pathogens lacking Avr genes leads to compatible interaction by allowing the pathogen to defeat host defense mechanism (Tyler, 2002). This 'gene-for-gene' interaction triggers a series of physiological changes at the site of infection, including programmed cell death known as the hypersensitive response.

Plants produce many important defense related proteins in response to elicitor treatment. These pathogeneses related (PR) proteins are polypeptide with relatively low molecular weights (10,000-40,000) that accumulate extracellularly in infected plant tissue and exhibit high resistance to proteolytic degradation. PR proteins are acid-soluble, protease resistant proteins that are antifungal

and directly act on fungal cell wall to weaken fungal structures. PR proteins have been classified into 17 families, denoted PR-1 to PR-14, in varying amounts detected in tobacco, tomato, cucumber, parsley, radish, Arabidopsis, and barley for example, peroxidase (PR-9), chitinases (PR-3, PR-4, PR-8, PR-11) and a β-1,3-glucanase (PR-2).

Another defense strategy employed by the plant is popularly known as the "mustard bomb". When plant tissues are chewed and physically damaged by the insects, the enzyme myrosinase activated in damaged plant tissue and converts the glucosinolates to a number of compounds including thiocyanates, nitriles and isothiocyanates which are harmful for the pests. Glucosinolates and their breakdown products have long been known for their fungicidal, bacteriocidal & nematocidal properties. Induced resistance in plants against biotic stresses is also attributed to the phenylpropanoid and octadecanoid pathways mediated by salicylic acid (SA) and jasmonic acid (JA), respectively. These pathways produce a number of plant defensive secondary metabolites in intermediate steps, which affect insect growth and development and also release volatiles that attract the insect's natural enemies. These HIPVs (Herbivore induced plant volatiles) provides the direct defense of the plants where plant is directly affecting the insect growth and development through toxic secondary metabolites. Other strategies include release & deposition of toxic furanocoumarins, toxic amino acids, trichomes, lignin and latex on the outer surface of plants.

## CONCLUSION

Mustard is often attacked by pathogens like *A. candida*, *P. parasitica* & *S. sclerotiorum*, *A. brassicae*. The plants are sprayed with chemicals like iprodione, ridomil, carbendazim, quinalphos etc to control these pathogens. Phytoalexin molecules, however, are plant's natural defense barrier to fight with these pathogens. Brassilexin, cyclobrassinin, cyclobrassinin sulfoxide, indole-3-acetonitrile, spirobrassinin, sinalexin&rapalexin are some of the phytoalexins present in mustard. They are byproducts of shikimic or mevalonic acid pathways & have cytotoxic or antiproliferative defense action. The defense strategy against insects include release of toxic metabolites or recruitment of natural enemies of the insect pests.

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