Downy Mildew of Sunflower (*Plasmopara halstedii*), a Disease Introduced and Established in India – Status Report in Relation to Quarantine

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Downy mildew of sunflower (*Helianthus annuus* L.) caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni is potentially a destructive disease. It is distributed almost in all the sunflower growing countries. The disease incidence range from traces to 50%, however, it may exceed up to 95%. In India, downy mildew of sunflower was reported for the first time in 1984 from Latur district of Marathwada region of Maharashtra; it has spread to Karnataka, Andhra Pradesh, Tamil Nadu, Punjab and Madhya Pradesh. The status of the disease world over, physiological races, host range, epidemiology, detection techniques and management is discussed.

Key words: Sunflower, Downy mildew, *Plasmopara halstedii*, Distribution, Races, Management, Quarantine

Sunflower (*Helianthus annuus* L.) is an important oil-yielding crop, which suffers from several diseases. In India, the commercial cultivation of sunflower has become extremely popular in recent years in many states. Simultaneously the common diseases attacking the crop were restricted to rust, *Alternaria* blight, head rot and charcoal rot. Downy mildew of sunflower caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni is potentially a destructive disease. In Europe, after its first appearance in 1941 the disease increased rapidly and by 1977 it was rated a "major disease" in all sunflower-producing countries (Sackston, 1981). In India the occurrence of downy mildew was reported first time in 1984 from Latur district of Marathwada region of Maharashtra (Mayee and Patil, 1986) and subsequently it spread to several states.

The disease is seed and soil-borne and causing considerable economic losses. In western Canada, the incidence of downy mildew increased from 6% in 1988 to > 20% in 1990 and 1991 (Rashid, 1993). In Morocco, Serrhini *et al.* (1994) reported that the incidence of downy mildew ranges from 1 to 40%. In India also, the disease incidence was reported up to 40% in farmer's field in Marathwada region of Maharashtra (Chattopadhyay *et al.*, 1999). A loss of more than 80% of production was also reported due to downy mildew in Spain (Molinero-Ruiz *et al.*, 2003a).

Zimmer and Zimmerman (1972) reported that seeds from plants infected with *Puccinia helianthi* and *Plasmopara halstedii* were significantly smaller, lighter, higher in hull percentage and lower in oil content. Doken (1989) observed that sunflower seeds produced in downy mildewed plants were either under-developed, colourless or, rarely, they look healthy. Such infected seeds were of poor quality, produced abnormal seedlings and low rate of germination.

Geographical Distribution

Plasmopara halstedii has been reported from Albania, Argentina, Austria, Azerbaijan, Bosnia, Brazil, Bulgaria, Canada, Chile, China, Czech Republic, Egypt, Estonia, Ethiopia, France, Germany, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Japan, Kazakhstan, Kenya, Mexico, Moldova, Morocco, Pakistan, Paraguay, Poland, Romania, Russian, Siberia, Slovakia, Spain, Switzerland, Ukraine, Yugoslavia, Georgia, Turkey, South Africa, Uganda, Zimbabwe, Dominican Republic, United States of America, Uruguay, Australia. In New Zealand, it is absent but formerly reported as present (Hall, 1989a; Kucmierz, 1976; CMI, 1988; EPPO, 2003, CABI, 2004).

Introduction and Spread in India

In India, it was reported for the first time in 1984 from Latur district of Marathwada region of Maharashtra (Mayee and Patil, 1986) and by 1989-90 the disease spread to western Maharashtra and Vidharbha in Maharashtra and further southern states (Patil *et al.*, 1993). It has also been reported from Andhra Pradesh (Moses, 1989) Punjab (Thind *et al.*, 1999), Karnataka (Patil *et al.*, 1993), Madhya Pradesh (Agrawal *et al.*, 1991) and Tamil Nadu (Chattopadhyay *et al.*, 1999).

Host Range

Main host: Cultivated sunflower (*Helianthus annuus*)

Other hosts: *Senecio* sp., (Gullino and Garibaldi, 1988)

Asteraceae: *Artemisia vulgaris* (Viranyi, 1984)

Wild species of *Helianthus*: *H. argophyllus*, *H. debilis*

H. divaricatus, H. grosseseratus and *H. petiolaris* (Viranyi, 1984)

Other wild hosts: *Xanthium strumarium* (Viranyi, 1984)

Common ragweed (*Ambrosia artemisifolia*) (Walcz *et al.*, 2000; Vajna, 2002)

Marshelder (*Iva xanthiifolia*) (Gulya, 2002)

Taxonomy and Nomenclature

Plasmopara halstedii belongs to Domain: Eukaryota; Kingdom: Chromista; Phylum: Oomycota; Class: Oomycetes; Order: Peronosporales; Family: Peronosporaceae (CABI, 2004).

The fungus is obligately biotrophic. Mycelium composed of intra-cellular, colourless, aseptate, hyphae 6-20 μm diameter, often irregularly shaped and swollen, bearing small rounded vasicular haustoria 5-10 μm diameter, growing in all plant tissues. Sporangiophores hypophallous or very occasionally epiphyllous, arborescent, 300- 750 μm x 7-14 μm, obconical and pointed at the base, branching in the upper half with the apex of the branching axis, which is frequently swollen; branches in the form of a whorl of 7-8 produced monopodially at right angles to the main sporangiospore axis, each with 2-5 secondary branches 40-86 μm long, bearing 3-5 tips, 8-15 μm long, diverging at right angles. *Plasmopara* is chiefly distinguished from other downy mildew genera by slender sporangiophores, which branch monopodially, usually at right angles and abtuse tips of the branches. Sporangia ovoid to elliptical, 18-30 x 14-20 μm, papillate germinating to give 20 reniform, biflagellate zoospores released by each sporangium. Antheridia club shaped, 12 x 30 μm, formed on distant hyphal branches ('diclinous'). Oogonia spherical, 30-40 μm diameter, colourless. Oospores formed in all the vegetative organs of the host, especially in roots and leaves, just under the epidermis, spherical, 23-30 μm diameter ('aplerotic'), yellow-brown with a slightly wrinkled wall, 3 μm thick, germinating to give sporangia (Hall, 1989a).

Sexual reproduction is by means of oogamy; oogonia, spherical in shape and club-shaped antheridia form on distal hyphal branches separately, those hyphae being of different mating types. Oospores are spherical, hyaline to light brown, thick-walled, temporarily surrounded by oogonial remnants (Hall 1989b).

Originally the fungus causing downy mildew was described as *Peronospora halstedii* by Farlow in 1879 who first collected it from *Eupatorium purpureum* but after a revision of the genus *Peronospora*, the fungus was renamed as *Plasmopara halstedii* (Farl.) Berlese and de Toni. In 1888 it was put correctly as *Plasmopara halstedii* (Farl.) Berl. & de Toni by P.A. Saccardo (*Sylloge fungorum* 7: 242). A number of forms and varieties have been separated from *P. halstedii* and described as a new species by several workers (Leppik, 1966). Novotelnova (1966) described three *forma species* within the fungus on *Helianthus* (1 on annual and 2 on perennial sunflowers) and put the downy mildew fungus to specific rank as *Plasmopara helianthi* Novot., a name widely used in Eastern Europe while in the western world *P. halstedii* is preferred. The taxonomic position is based on morphological characteristics and there is as yet no precise work on developmental biology and physiology to define the pathogen species (Mayee, 1988).

It is important to note that in India, *Plasmopara halstedii* downy mildew has been reported on *Veronia cinera* at Allahabad, Uttar Pradesh (Edward and Naim, 1962). This appears to be a different form than the one occurring on cultivated sunflower and can be raised to the level of species after proper investigation. On *Veronia chinensis* also, a downy mildew caused by *P. veronia-chinensis* Less was reported from Hyderabad and further South (Salam and Rao, 1954; Ramakrishnan and Sundaram, 1954). A perusal of literature indicated that *P. halstedii* is a complex pathogen and is highly specialized, probably several species, forms or varieties exists in it that adapted on to certain host species. The grouping therefore, is yet incomplete and needs to generate more information on the taxonomic position in India (Mayee, 1988).

Race Situation

Zimmer (1974) found evidence for existence of races. Sunflower lines that were found resistant in the USA were also resistant in Europe but vice-versa it was not true. These studies indicated the existence of physiologic races in *P. halstedii*. The existence of pathogenic forms within *P. halstedii* became evident soon after the release of the first mildew-resistant sunflower cultivars. Initially, two pathotypes (races) of the fungus were differentiated: pathotype 1, originally referred to as the European race, and pathotype 2, known as the Red River race (referring to the Red River Valley of North America). By now, over 20 different races/pathotypes of *P. halstedii* have been recorded and several workers suggested new systems for their classification. Gulya *et al.* (1991a) mentioned

the standard method to determine races of sunflower (*Plasmopara halstedii*) and differentiated 8 races in USA. Accordingly, Labrouhe and de Labrouhe (1999) applied a new nomenclature to French type of *P. halstedii* and renamed the prevalent races. Existence of races have been reported from Argentina, Bulgaria, Canada, France, Germany, Hungary, Italy, Morocco, Pakistan, Romania and Spain (Pacureanu *et al.*, 2002; Labrouhe and de Labrouhe, 1999; Gulya *et al.*, 1991b; Rashid, 1993; Zazzerini and Tosi, 1998; Kormany and Viranyi, 1997; Achbani *et al.*, 1996a; Rahim *et al.*, 2002; Molinero Ruiz *et al.*, 2002; Rozynek and Spring, 2000). In India also, consistent resistant reaction of three cultivars *viz.*, RHA-265, RHA-273 and RHA-274 and susceptible reaction of cv. Progress confirmed the identity of Indian isolates as European Race-1 (Patil and Mayee, 1990).

Symptoms on Host Plant

The symptoms produced by *P. halstedii* in sunflower are diverse depending on the age of tissue, level of inoculum, cultivars reaction and environment (moisture and temperature). The variety of symptoms produced by the pathogen is damping off, basal root or stem galls, local foliar lesions, latent infection and systemic symptoms.

A. Damping off

Infected seed when sown in favourable weather conditions particularly under cool and moist conditions showed damping off symptoms in the seedling stage. Seedlings are killed soon after the emergence resulting into reduced plant population. Affected plants become dry and easily blown by winds.

B. Basal Root or Stem Galls

Development of basal gall symptoms occurs independently of the infection that results in systemic symptoms. Root infection may result in formation of root galls at the base of the plants on primary roots. Such roots become discoloured, scurfy and hypertrophied. Plants ultimately show the wilt symptoms. Some time lodging of the plants also occur from the point of infection. Oospores are formed on the affected tissue. Plants with basal galls are more prone to drought stress and lodging than healthy plants.

C. Local Foliar Lesions

Small angular chlorotic spots appear on the leaves as a result of secondary infection through zoospores liberated from wind borne zoosporangia. Spots may coalesce to form a larger patch. These spots or patches show white fungal growth on the undersurface of the leaves usually under high humid conditions. During morning hours it becomes more conspicuous. Chattopadhyay *et al.*, (1999) reported whitish downy growth, chlorosis, stunting, thickening and curling of leaves, erect flower heads, chlorotic local lesions and a reduction of the root system. In Hungary, Vida (1996) reported a local- to systemic infection in some sunflower fields due to favourable weather conditions coupled with unusually high infection pressure.

D. Latent Infection/Symptom

The latent type of infection appears symptomless and affected plants cannot easily be recognized from outside. It may derive either from below ground infections on plants or it may derive from very late infections during flowering stage, when the growth of vegetative parts has finished and therefore no symptoms become visible. The former type is typical and appears in some so-called resistant sunflower genotype (which sometimes allow sporulation on hypocotyls and cotyledons) and the latter mostly leads to seed contamination. In both cases the pathogen stays alive and is able to complete its life cycle through sexual reproduction by the formation of oospores. Plants with latent infections showed enhanced cell division, which restricted the pathogen to the parenchyma (Heller *et al.*, 1997).

E. Systematic Symptoms

In case of systemic symptoms plants become severely stunted. Leaves of systemically infected plants show abnormally thick, downward curled with yellow and green epiphyllous mottling. The chlorosis usually starts from the midribs. Hypophyllous downy growth of the fungus consisting of zoosporgiophores appears and sporangia develop on them. Stem becomes brittle. Systemic infection occurred more readily through hypocotyls than roots and through apical buds than leaves. Sporangial infection caused local lesions on young leaves and some of these infections become systemic (Cohen and Sackston, 1973a). Severely infected plants do not produce any seeds in the heads.

Epidemiology

The nature of the inoculum (oospore or zoospore), weather variables (relative humidity, temperature), infection site (age of tissue), as well as cultivar reaction are the main factors, which determine the infection process, disease incidence, and severity. Zoospores,

originating from either sexual or asexual sporulation, require free water for retaining viability and capability of moving towards infection sites. The symptoms become visible in the seedlings within a week of emergence. Seedlings become stunted and abnormally thick; downward curling of cotyledons and epiphyllous mottling on the true leaves. Severely infected seedlings often succumb to disease or may survive for few weeks. Such type of infection is systemic and results from penetration of the host at an early stage of growth up to the 6-8 true leaf stage. Raghuvanshi *et al.* (1999) reported that rainfall immediately after sowing coupled with high RH and favourable temperature were the main factors affecting downy mildew incidence irrespective of sowing dates. Chattopadhyay and Appaji (2000) observed the maximum growth and sporulation of the fungus at 16±1^{0}C and at 100% RH with no effect of light hours. They also reported that infectivity of the pathogen was negatively correlated with age of seedlings and temperature while a positive correlation was found between humidity and growth/ sporulation of *P. halstedii*. Kormany and Viranyi (1997) found that as little as 20-25 sporangia/ ml suspensions produced sufficient and comparable disease symptoms in sunflower. Secondary infection by wind-borne zoosporangia formed on the leaves.

Brief Life Cycle

In the presence of free water, the zoospores remain in motion for hours and tend to move to infection sites (root, hypocotyl) soon if available. Following encystment and germ tube elongation (the latter usually terminates in an appressorium against a host cell) the fungus develops an infection structure (infection peg) for direct penetration. Gray *et al.* (1985) demonstrated that germ tubes do not usually form appressoria in water but they do so in the presence of host cells. After penetration, the fungus grows intracellularly and then intercellularly, and once being established in a susceptible host (compatible) it starts to colonize the entire plant systemically by growing preferably towards the shoot apex and, to a lesser extent, in the direction of the root. In favourable conditions asexual sporulation takes place on affected leaves and occasionally on below-ground tissues. Fully developed sporangia disseminate by wind and their survival depends on the current weather situation. Oospores are also produced in infected plant parts, primarily in root and lower stem tissues, whereas leaves and upper plant parts, except seeds, are free from these spores (Viranyi, 1988; Onan and Onogur, 1991).

Spring (2000) observed the formation of oospores in the host tissue of all plants infected with single zoospore, which indicated the homothallic nature of sunflower downy mildew. Initiation of sexual reproduction started two to three week after inoculation. The oogonia and antheridia developed in close proximity to each other at the same hypha (Spring, 2000).

Source of Inoculum, its Survival and Transmission

P. halstedii is a seed as well as soil-borne pathogen. Soil-borne oospores serve as primary inoculum to underground tissues of young sunflower seedlings. Oospores develop mainly in root and lower stem tissues of mildewed plants, with or without visible symptoms and, with plant residues of the preceding sunflower crop, they come into the soil. Oospores can remain viable for at least 6-8 years (Sackston, 1981; Virányi, 1988). The spore viability in the soil decreased only after 5-6 years, sometimes after 8-10 years (Vronskikh, 1981).

Infected seeds carry mycelium and/or oospores internally. Sackston (1981) confirmed the seed infection by artificial inoculation. Such infected seeds gave rise to apparently healthy seedlings with no typical symptoms (latent type of infection) but the pathogen sporulates more often during flowering stage on the roots of these symptomless plants. Spring (2001) described the crucial role of such symptomless and non-systemic infections for the distribution of the pathogen.

P. halstedii may also be wind-borne and causes secondary infection of leaves and/or inflorescence. The secondary infection of inflorescence may give rise to latent infection of seeds (Sackston, 1981). Vida (1996) reported that secondary infection by zoosporangia was numerous in some Hungarian fields in 1995 and 1996. The secondary infection is considered as an important factor in the spreading of the disease in certain regions under favourable environmental conditions.

Seed Borne Aspects

Plasmopara halstedii has been found to occur in sunflower seeds from naturally infected plants, either as mycelium or oospores (Novotelnova, 1966). Cohen and Sackston (1974) reported that the pathogen may be present with the seed in three ways i.e. oospores on the surface of the seed; oospores in internal parts of the seed; and mycelium in endosperm and embryo. Cohen and Sackston (1973b) confirmed that sunflower buds inoculated with *P. halstedii* became systemically infected and produced infected seeds at 15-30^{0}C. Oospores were

observed in seeds of inoculated and naturally infected plants in the field. Basavaraju *et al.* (2004) detected the oospores in seed coat and endosperm of systemically infected seeds and confirmed seed transmission of *P. halstedii* in sunflower. However, Doken (1989) reported that the mycelium was found only in the testa and inner layer of the pericarp; it was absent from the embryo. Seed infection regularly occurs in systemically infected plants if they survive up to the flowering stage.

Seed Health Tests

Different techniques for the detection of *Plasmopara halstedii* are as follows:

A. Seed Washing

Basavaraju *et al.* (2004) successfully detected the surface borne oospores of *P. halstedii* in infected seeds by washing test. They took 100 seed from each sample which were immersed in 20 ml of distilled water separately with a drop of Tween 20 and then shook for 10 min. The supernatant solution was discarded and the sediment was suspended in 2 ml of sterile distilled water. On examination of suspension from each samples under light microscope, they successfully detected the surface borne oospores in infected samples.

B. Seed Maceration Technique

Basavaraju *et al.* (2004) detected the fungus in seed and seed components also. They soaked 400 seeds from each sample in a solution of 5% of NaOH (v/v) mixed with Tryptophan blue (0.02% w/v) and incubated for 4 hour at room temperature (the optimum concentration of NaOH and the period for soaking they found). Subsequently, seeds were washed with hot water with agitation to separate different seed parts from each other. The seeds were dehydrated with 70% alcohol and boiled in lectophenol. The material was then transferred to fresh lactophenol. On examination under stereobinocular microscope, they detected the oospores and fungus hyphae of *P. halstedii* in both seed coat and endosperm of systemically infected seeds. They observed the fungus in the cotyledonary surface, plumule, radicle and seed coat also and suggested that the technique is simple and more precise, could be used for detection of *P. halstedii* in sunflower seeds at quarantine stations and even during shipment itself.

C. Blotter Test

Seeds were surface-sterilized, rinsed and placed on 3-4 layers of wet filter paper and were incubated at 25-30^{0}C to germinate them. After a few days of incubation when roots are formed, the fungus if present will sporulate on their surface under humid conditions, which can be observed under stereobinocular microscope. The slides can also be prepared and examined under compound microscope, if necessary. This sporulation may also occur with otherwise symptomless, latent infected plants (Gulya, 1995).

D. Molecular Techniques

P. halstedii can also be detected by immunological tests (Bouterige *et al.*, 2000a) and Polymerase Chain Reaction (PCR) techniques (Roeckel-Drevet *et al.*, 1999). Bouterige *et al.* (2000b) produced two monoclonal antibodies (MAbs) i.e. 12 C 9 and 18 E 2 by immunizing mice with a partially purified extract of *P. halstedii* race 1. Both Mabs detected all races of *P. halstedii* present in France in enzyme-linked immunosorbent assay (ELISA). On inoculation of seed homogenates from infected plants in wells coated with Mab 18E2 resulted in trapping of *P. halstedii* antigens that were identified with biotinylated Mab 12C9. No reaction was observed with seed homogenates from healthy plants. They suggested that these Mabs might be used to develop a sandwich ELISA detection system for detection of fungus in infected seeds of sunflower.

Roeckel-Drevet *et al.* (1999) developed a rapid assay to detect infection of *P. halstedii* in sunflower. Based on the nucleotide sequence information, they designed specific oligonucleotides and used as primers for *in vitro* DNA amplification by PCR. They detected an amplification product on agarose gel stained with ethidium bromide when DNA from various *P. halstedii* races was tested, whereas no amplified DNA was detected when DNA from other origin was tested. The assay successfully reveals the presence of the fungus in infected plants prior to sporulation also.

E. Fatty Acid Analysis

Spring and Haas (2002) reported that fatty acid analysis might become a diagnostic feature for the contamination of sunflower seeds with downy mildew. They also reported that a fatty acid composition of *P. halstedii* revealed a characteristic profile which was different from profile of all other parasitic and pathogenic fungi of sunflower by the dominance of 5Z, 8Z, 11Z, 14Z, 17Z-eicosapentaenoic acid.

Quarantine Risk

Downy mildew of sunflower is seed as well as soil-borne disease. Seeds play an important role in the dissemination of downy mildew from one place to another. The perusal of literature indicated that more than 20 different races/ pathotypes are prevalent in *P. halstedii* world over. Ram Nath *et al.* (1981) intercepted oospores of *P. halstedii* on sunflower seeds imported from Bulgaria while processing for quarantine clearance. Therefore, there is an inherent risk of introduction of the new race or more virulent strain of *P. halstedii* in the country.

Precautions

1. Quarantine regulations should be enforced strictly to restrict the introduction of infected seeds in the country from other countries.
2. Exporting countries should be requested to provide phytosanitary certificates declaring that the seeds are free from downy mildew.
3. Importation of seeds through letter post should be completely prohibited.
4. In case the research workers receive the seed samples directly from abroad they must send the sample to quarantine authority for quarantine clearance.
5. The imported seed should be grown in isolation in the post entry quarantine nursery to reduce the danger of introducing new race/pathotype or more virulent strains.
6. The seeds should be treated with a suitable fungicide to overcome to the risk for spread of the pathogen by latent infection.
7. Domestic quarantine should be enforced strictly in the country to restrict the internal movement of infected seeds among the states.

Disease Management

A. Cultural Control

Muresan and Vranceanu (1972) reported that the incidence of downy mildew decreased with increasing length of rotation (6-7 course rotation) and the incidence was negatively correlated with yield. Jimenez-Diaz (1973) also reported that the incidence of downy mildew in Spain was 15.6% in plots where wheat and sunflower was grown alternatively whereas it was upto 100% in plots without crop rotation. In India also, the disease intensity increased with continuous cultivation of sunflower in the same field (Mayee, 1988). He suggested the cultivation of an alternate crop as per suitable agro-system, roguing of mildewed seedlings during thinning, removal and destruction of diseased plants before flowering by burning and to avoid the seed multiplication in affected areas so as to get the seed from a disease free area for sowing purposes.

Early sowing of crops also suppressed downy mildew incidence and resulted in higher yields than crop sown in the season (Weldekidan, 1986). However, Patil *et al.* (1992a) reported that sowing dates had no effect on sunflower downy mildew in India but frequent rains immediately after sowing play an important role in the disease development and sunflower seedlings become resistant with age to the downy mildew infection. Application of 400 kg ammonium nitrate (AN) or 200 kg AN + 200 kg super phosphate/ha to sunflower also decreased local infection of mildew and increased the seed yield (Grinberg, 1976).

B. Chemical Control

Seed treatment with large number of protectant chemicals such as organo mercurials and thiram did not give encouraging results in France and Soviet Union. Szoko (1973) reported that seed dressing with vitavax 75 [carboxin] @ 500 g/100kg seed reduced infection from 30% to 50%. In Canada, Morocco, Romania, Turkey and former USSR metalaxyl (Ridomil 25 WP or Apron 35 SD) provided complete protection against the disease when the seeds were treated with the chemical @ of 3 to 6 kg/kg seed (Achbani *et al.*, 1996a;). In Spain, seed dressing with metalaxyl (1, 2 or 4 g a.i./kg) prevented *P. halstedii* sporulation on seedling inoculated by dipping root into a zoosporangial suspension (Melero-Vara *et al.*, 1982). In India also seed treatment with metalaxyl (as apron 35 SD) @ 6g/ kg seed gave the best control of downy mildew. Rhodex (fosetyl-aluminium + mancozeb) and fosetyl-aluminium also gave effective control of the disease (Patil and Mayee, 1988; Patil *et al.*, 1991). Chattopadhyay *et al.*, (1999) also reported that three foliar applications of metalaxyl + mancozeb also controlled the disease. However, Molinero-Ruiz *et al.* (2003a) reported the resistance of sunflower downy mildew to metalaxyl while studying the effect of seed treatment with metalaxyl at 0.5, 2.0 and 3.5 g a.i./kg seeds. Tosi *et al.*, (1999) reported that a novel synthetic chemical CGA-245704 [benzo (1.2.3) thiadiazole-7-carbothioic acid S methyl ester (acibenzolar-S-methyl) (BTH)] when applied as a soil drench @ 150 and 200

mg/ kg soil provided 80-82% protection from the disease. Application of CGA-245704 as soil drenching and foliar spray also protected sunflower plants from *P. halstedii*.

C. Host Resistance

The best method for control of downy mildew is the cultivation of resistant varieties. But the disease resistance may break down once the pathogen produces more virulent races. It is relatively easy to produce mildew resistant hybrids by crossing a cytoplasmically male sterile line with a fertility restoring line, if one of the parents is homozygous for resistance. Nine different genes Pl_1, Pl_2, Pl_3, Pl_4, Pl_5, Pl_6, Pl_7, Pl_8, and Pl_9 have been reported as resistant to downy mildew in sunflower by different workers (Sackston *et al.*, 1990). Vear and Leclarcq (1971) reported two independent dominant genes also (H1 and H2) from an American source of resistance to *P. halstedii,* which were different from Pl gene. Presently the Pl gene is being used to give mildew resistance in breeding programme in many parts of the world. A large number of resistant lines/ cultivars have been reported as resistant to downy mildew included from USA, Canada, France, Italy, Romania, Spain, Yugoslavia and Russia (former USSR) (Gulya *et al.*, 1991b; Seiler, 1993; Dedio and Rashid, 1994; Mouzeyar *et al.*, 1994; Molinero-Ruiz *et al.* 2003b;). In India also a number of sunflower cultivars possessing resistance to downy mildew have been reported (Patil and Mayee, 1988, 1990; Patil *et al.* 1992b; Manjula and Seetharam, 2000; Dandnaik and Deshpande, 2003).

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