J. Mycopathol. Res. ***53(1)*** *: 9-24, 2015;* (ISSN 0971-3719)

REVIEW

Important tobacco diseases in India and their management

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Received : 23.06.2014 RMS Accepted : 15.12.2014 Published : 27.04.2015

Tobacco is one of the important cash crops in India earning a sizeable foreign exchange, internal excise revenue and generating employment to many people. It is grown in almost all the states in India, traditionally in black-soils though its cultivation is now extended considerably to light soils. Cultivated tobacco coming under *Nicotiana tabacum* L. and *Nicotiana rustica* L. is susceptible to several fungal, bacterial, viral and nematode diseases. These diseases not only reduce the yield of tobacco but also impair the quality parameters of the cured leaf. In normal years average crop loss due to diseases is estimated to be 5 to 10 per cent. In the present article the disease scenario of important nursery and field diseases like damping off (*Pythium aphanidermatum*), black shank (*Phytophthora parasitica* var. *nicotianae*), brown spot (*Alternaria alternata*), frog eye leaf spot (*Cercospora nicotianae*), sore shin (*Rhizoctonia solani*), bacterial wilt (*Ralstonia solanacearum*), hollow stalk (*Erwinia carotovora* sub sp. *carotovora*) and tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), tobacco leaf curl virus (TLCV) and broomrape (*Orobanche* spp.) has been discussed based on work carried out at Central Tobacco Research Institute (CTRI), Rajahmundry, Andhra Pradesh and other research stations in India and abroad.

Key words: Tobacco, diseases, management, review

INTRODUCTION

Flue Cured Virginia (FCV) tobacco is one of the important cash crops in India earning a sizeable foreign exchange, internal excise revenue and generating employment to many people. It is grown in almost all the states in India, traditionally in black-soils though its cultivation is now extended considerably to light soils. In India tobacco is grown as important commercial crop in an area of 0.45 M ha which accounts for 0.27% of net cultivable area in the country with 750 M kg production (Krishnamurthy and Narasimha Rao, 2010). India stands second in tobacco production and exports in the world.

Cultivated tobacco coming under *Nicotiana tabacum* L. and *N. rustica* L. is susceptible to several fungal, bacterial, viral and nematode diseases. These diseases not only reduce the yield of tobacco but also impair the quality parameters of the cured leaf. In normal years average crop loss due to diseases is estimated to be 5 to 10 per cent. Since tobacco is grown as a monoculture, year after year in the same traditional belt which provides ideal conditions for gradual development and

perpetuation of pathogen causing serious disease problems under favourable weather condition during its active growth period. Leaf being the economic end product in tobacco, any blemish due to pathogen results in lowering the market value. Considerable research, both fundamental and applied on some of these diseases has been carried out since last 50 years at the Central Tobacco Research Institute, Rajahmundry, with special emphasis on evolving suitable control measures and resistant varieties to combat them. Details of research work on various aspects of different diseases are compiled in this review.

A. *Fungal Diseases*

Damping off

Damping off is one of the most important diseases of tobacco, especially in nurseries, showing decay of stems of young seedlings. This is responsible for poor stand of seedlings or complete loss of nursery beds.

Symptoms

The disease appears in the nurseries any time upto 35 days after sowing. It may appear soon after seedling emergence (pre-emergence damping off) and may be confused with poor germination. In early stage, tiny seedlings seem to disappear due to rotting causing daily reduction in seedling stand. Older seedlings show shriveling and dark brown discoloration of stem at the base and ultimately collapse and topple over. The wet rotting and collapse of seedlings start in circular patches and may extend to the entire bed, if unchecked.
The disease attacks the root region or stem region near the soil surface. Under humid conditions, white cottony growth of the pathogen appears on the infected seedlings and seedlings gradually dry off and become papery white in colour and responsible for as much as 90% death of seedlings (Fig.1A).

Etiology

This disease is mainly caused by a soil-borne facultative parasite, *Pythium aphanidermatum*, belonging to the family Pythiaceae, under class Phycomycetes but other species such as *Pythium myriotylum* may also be involved to cause damping off disease in tobacco nurseries (Anon., 2012). This organism is the most common in India and other countries, though several other fungi like *Phytophthora*, *Sclerotinia*, *Sclerotium, Rhizoctonia* and other species of *Pythium* have been reported to cause damping off disease in tobacco nurseries (Lucas, 1975).

Over-crowding of seedlings and presence of excessive organic matter predispose the seedlings to damping off disease.

Disease cycle

This fungus is primarily a saprophyte and in the presence of suitable host, it becomes a facultative parasite on the young roots, stems or any decaying organic matter. The fungus normally survives in the form of oospores or chlamydospores. Exudates from roots of germination seeds or actively growing seedlings stimulatethese resting structures, which germinate by germ tube or form zoospores. These zoospores or hyphae infect the young seedlings at the soil surface and the fungus proliferates fast. The secondary spread occurs during wet weather by means of surface or drainage water. The pathogen produces oospores which are released into the soil, when the infected plants decay.

Optimum conditions under which the fungus thrives better were reported to be temperatures below 24^0 C with a high relative humidity of > 85%, soil pH 5.5 to 7.5 and high soil moisture. Such conditions prevail as a result of high seed rate, continuous rain and cloudy weather during nursery season (Mathrani and Murthy, 1965).

Disease management
Cultural and preventive measures

a) Selection of nursery site,b) Deep summer ploughing,c) Raising the seed beds,d) Rabbing seed beds,e) Optimum seed rate and f) Regulation of watering.

Chemical management

Drenching nursery beds with Bordeaux mixture @ 1.0% before sowing and @ 0.4% after sowing 4-6 times at 3-4 days intervals was recommended earlier (Mathrani and Murthy, 1965; Pillai and Murthy, 1967). Mathrani and Murthy (1965) observed that drenching (0.2%) or spraying (0.4%) either Bor-

deaux mixture or any of the readily available copper fungicides for the control of damping off disease.

Spraying Ridomil (Metalaxyl + mancozeb) 25 WP twice @ 0.1, 0.2 and 0.3% concentrations were highly effective in controlling damping off disease (Nagarajan and Reddy, 1980 and 1982).

Shenoi and Abdul Wajid (1988 and 1992) observed that the chemical Ridomil (Metalaxyl + mancozeb) 72 WP @ 0.02% drenched before sowing and subsequently at 20 to 30 days after sowing (DAS) or, subsequently drenched with Bordeaux mixture (1.0%) at weekly intervals after every rain received and foliar spray of Ridomil (Metalaxyl + mancozeb) 72 WP @ 0.2 % concentration at 30 days after sowing (DAS) effectively controlled the damping off disease.

New molecules like Folio gold (Metalaxyl - M + chlorothalonil) 440 SC, Ridomil gold (Metalaxyl - M + mancozeb) 68 WP @ 0.2% concentration at 20 and 30 DAS showed superiority over other fungicides but at par with Ridomil (Metalaxyl + mancozeb) 72 WP @ 0.2% in controlling the damping off disease (Raju and Dam, 2011). Sectin (Fenamidone + mancozeb) 60 WG @ 0.3% (Raju *et al.*, 2014) and Amistar (Azoxystrobin) 23 SC @ 0.1% (Anon., 2012-13) concentration at 20 and 30 DAS showed superiority over other fungicides in controlling the damping off disease.

Timely application of fungicides is a prime factor in the disease management.

Biological management

Incorporation of inoculums of *Trichoderma harzianum* in artificially infested soil significantly reduced damping off disease. Use of metalaxyl treated seeds coupled with application of *T. harzianum* inoculums gave excellent control of the damping off disease under greenhouse conditions (Mukhopadhyay *et al.*, 1986).

Sreeramulu *et al.* (1998) observed in the nursery that the dual inoculation of VA mycorrhiza *Glomus fasciculatum* and *Trichoderma harzianum* was more effective in controlling damping off disease than the individual inoculation and resulted in better germination count and improved the plant growth parameters in comparison with Copper oxychloride 50 WP (0.2%) and metalaxyl MZ 72 WP (0.2%).

2. *Leaf blight and Black shank*

Among the major diseases of tobacco in India, leaf blight and black shank are important which occur both in nursery and field planted crop. It is soil borne and wide spread in Karnataka, Andhra Pradesh, Bihar and Gujarat states. Loss in yield due to this pathogen varies from 2 to 10 per cent annually depending on weather conditions. This disease is caused by *Phytophthora parasitica* f. sp. *nicotianae* (van Breda de Hann).

Symptoms

Young tiny seedlings are affected in the nursery, they rot and die suddenly. Seedlings show blackening of roots and stem at ground level. Under continuous wet weather conditions, large circular to irregular water soaked patches appear on the leaf surface causing leaf blight (Fig. 1B). Symptoms of black-shank on the transplanted tobacco are seen in the form of blackening of roots and stalk. Blackening of the stalk starts at the base near the soil gradually extended upwards upto 30 cm or more (Fig. 1C). The leaves turn yellow and the whole plants wilt and die. When the stem is split open, the pith is found dry, brown to black in colour and separated into plate like discs.

Favourable conditions and spread of the disease

Heavy rainfall after planting, continuous wet weather, temperature below 22^0C and high moisture lead to severe incidence. The fungus remains viable in soil for several years and possibly over winters as mycelia in dead tobacco stalks and roots. It spreads through irrigated water or through the air-borne sporangia, splashed during the rain.

All types of tobacco viz., Cigarette, Cigar, Natu, Bidi, Chewing, Cheroot and snuff are susceptible to this pathogen in India.

Disease management
Cultural and preventive measures

1.Deep ploughing in summer reduces the fungus inoculum level in the soil.
2.Raising of seed beds 15 cm high with channels around to provide drainage.
3.Rabbing the seed bed before sowing with slow

burning farm waste materials like paddy husk, tobacco stubbles, waste grass etc.
4.Use of seed rate @ 3.5 kg/ha only to avoid overcrowding of seedlings.
5.Regulation of watering to avoid excessive dampness on bed surface.

Chemical management

Spot drenching of Bordeaux mixture (5:5:500) or 0.2% Copper oxychloride 50 WP, Cuman or Dithane Z-78 for the control of black shank of tobacco (Anon., 1951-52 and Nagarajan *et al.,* 1977)

Gupta and Patel (1979) observed that Bayer 5072, Copper oxychloride 50 WP, Blue Copper 50, Bordeaux mixture (6: 3: 100), Burcop, Copper Sulphate, Fytolan, Macuprax, Miltox, Ziram, Daconil 2787, Difolatan, Dithane M - 45, Lonacol and Rovral exhibited *in vitro* and *in vivo* inhibition against *Phytophthora parasitica* var. *nicotianae* of tobacco.

Nagarajan and Reddy (1980) indicated that the most promising fungicide for the control of leaf blight disease was metalaxyl MZ 25 WP @ 0.1, 0.2, & 0.3% concentrations with high transplantable & total healthy seedlings. Elias *et al.* (1981) revealed that one spray of metalaxyl MZ 25 WP at 10 days after sowing effectively controlled the disease.

Shenoi and Abdul Wajid (1988 and 1992) showed that the chemical Ridomil (Metalaxyl + mancozeb) 72 WP @ 0.02% drenched before sowing and subsequently at 20 to 30 days after sowing (DAS) or, subsequently drenched with Bordeaux mixture (1.0%) at weekly intervals after every rain received and foliar spray of Ridomil (Metalaxyl + mancozeb) 72 WP @ 0.2% at 30 days after sowing (DAS) effectively controlled the black shank disease.

Single application of Metalaxyl MZ 72 WP in the planting hole along with planting water, @ 0.1 % (150 ml / hole) gave 100% control of black shank disease (Abdul Wajid and Shenoi, 1992).

Sources of resistance

.Growing resistant varieties is the best method of controlling the disease. Out of the 122 entries screened, only seven viz. Beinhart 1000-1, Coker 411, F 180, F 210, MC 1610, Reams 64 and Va 770 showed resistant reaction to all the three races of *P. parasitica* var. *nicotianae* (Abdul Wajid *et al.*, 1987).

Biological management

Sreeramulu *et al.* (1998) observed in the nursery that the dual inoculation of VA mycorrhiza *Glomus fasciculatum* and *Trichoderma harzianum* was more effective in controlling black shank diseases than the individual inoculation and resulted in better germination count and improved the plant growth parameters. Patel and Patel (1999) also observed that *Trichoderma harzianum* was highly antagonistic to race 'o' of *P. parasitica* var. *nicotianae* under *in vitro* conditions.

3. *Brown spot*

Brown spot of tobacco caused by *Alternaria alternata* (Fries) Keissler has wide host range. The target pathogen, attacks all kinds of tobacco i.e. FCV and non-FCV tobacco in India, and elsewhere in the world. Brown spot disease of tobacco, in general, is a disease of senescence.

Symptoms

The first indication of infection on the older leaves of the plant is small water- soaked lesions which enlarge quickly. As the spots enlarge, the centers die and become brown, leaving a sharp line of demarcation between diseased and healthy tissue. On the lower leaves, the brown spot lesions with concentric rings are mostly circular and usually range from 1-3 cm or more in diameter. On the upper leaves, the spots are usually smaller and range up to 1.5 cm in diameter. In severe infection spot enlarge, coalesce and damage large areas making leaf dark brown, aged and worthless (Fig. 1D).

Variability of the pathogen
Symptomatological variation of isolates

In *Motihari* tobacco (*N. rustica*), the spots produced by isolate Aa 1 were large (1.2 to 2.7 cm), round, dark brown in colour having concentric rings with prominent yellow halo and Aa 2 isolates were medium (0.3 to 0.5 cm) size having concentric rings having less yellowing and halo. Maximum coverage of leaf area was observed for Aa 1 isolate under field conditions in *Motihari* tobacco (Dam *et al.*, 2010a).

Growth Characteristics of isolates

Three different isolates showed differences in their

morphological variability and growth character (Dam *et al.*, 2010a). The isolates exhibiting higher degree of virulence had slower growth rate (10 mm/day) as compared to less virulent ones (11 mm/day). The observation is in conformity with Dong and Wang (1990) who observed that more virulent strains of *A. alternata* showed slower growth of colony, vigorous extension of aerial hyphae and less sporulation.

Morphological variability of isolates

Maximum conidial length (48) and beak length (12.07) were measured in isolate Aa 3 while in Aa 1 conidial length (26.0) and beak length (7.6) was lower. The study (Dam *et al.*, 2010a) indicated that higher the ratio of beak length to conidial length, the virulence of *A. alternata* was more on *Motihari* tobacco.

Virulence pattern

The virulence pattern of three isolates (Aa 1, Aa 2 and Aa 3) of the pathogen, *Alternaria alternata* collected from brown spot affected *Motihari* tobacco in North Bengal were inoculated on 35 day old plants of var. Dharla with spore suspension (10^5 spores/ml) in atomizer. Highest number of spots was observed on isolate Aa 3 but maximum necrotic area was observed in Aa 1 in observations made after 6 days of inoculation. From the perusal of the data, it is evident that isolate Aa 1 is more virulent and aggressive when compared to other two isolates of *Motihari* tobacco under *terai* agro-ecological region of West Bengal (Dam *et al.*, 2010a).

Dong and Wang (1990) obtained twenty strains from 10 different locations in China. The more virulent strains showed slow growth of colony, vigorous extension of aerial hyphae and weak sporulation, while it was inverse in case of weakly virulent strains.

Predisposing factors in relation to weather parameters

The studies (Dam, 2008) carried out in *terai* zone of West Bengal indicated the appearance of the disease during first week of February in the field and its intensity increased gradually up to the maturity of the crop. In the crop seasons 2005-06, the disease score was highest as per observations recorded during 11-18th March, 2006. Therefore, the weather factors like temperature (25.1^{o}C to 13.4^{o}C), relative humidity % (96 to 54), total rainfall (9.3 mm) and bright sunshine hours (7.4) prevalent during this period were favorable for rapid build-up and spread of the disease.

Sources of resistance

Nagarajan *et al.* (1984) tested 740 collections of *N. tabacum* and *N. rustica* against brown spot pathogen and none were found to be resistant although a few had shown slightly less degree of susceptibility. Monga and Dobhal (1987) tested 25 cigar wrapper tobacco lines against brown spot of tobacco and reported that no line was highly resistant. However, sixteen lines were in the resistant category while three and six lines fell in the susceptible and highly susceptible categories. Monga and Dobhal (1989) found that out of 121 hookah and chewing tobacco lines, most fell in moderately resistant to moderately susceptible groups.

Among the various *Nicotiana* species, only *N. debneyi* has shown immunity to brown spot, whereas *N. exiqua, N. glutinosa, N. longifolia, N. nesophila* and *N. plumibaginifolia* have shown resistance (Reddy *et al.*, 1976). Nagarajan and Shenoi (1998) observed that there were no resistant varieties of *N. tabacum* while resistant donor is available in wild *Nicotiana spp.* Three entries in accordance to rating scale developed by Shenoi *et al.* (2002) viz. Vaishali Special, PT-76 and Gandak Bahar were found to be resistant out of a total of 60 germplasm accessions of *Jati* tobacco (*N. tabacum*) screened for resistance to brown spot in North Bengal (Anon., 2007-08)

Disease management
Cultural management

Monga (1990) reported that intensity of disease in different dates of planting from northern part of West Bengal in India. Out of 4 different dates of planting, brown spot index was found to be lowest on 20th November compared to 6th November, 30th November and 15th December. Compared to other planting dates, the planting on 20th November registered higher productivity of quality leaf. Drastic yield reduction in late planting cigar wrapper (*Nicotiana tabacum*) tobacco in North Bengal has also been earlier observed by Srivastava and Subba Rao (1984).

Chemical management

Chari and Nagarajan (1994) suggested Dithane M-45 and Foltaf for brown spot control. Monga (1991) reported two sprays, one at disease appearance and another after ten days either with Thiram at 0.2% or Dithane M-45 at 0.2% were promising in reducing brown spot of *Motihari* tobacco with higher monetary returns. The fungicides, propiconazole (Tilt), difenoconazole (Score), Captafol (Foltaf) and Mancozeb (Dithane M-45) reduced disease incidence besides improving the yield (Murthy and Shenoi, 2001). Mahtabi *et al.* (2001) found propiconazole (Tilt), tebuconazole (Folicur) and Mancozeb (Dithane M-45) fungicides to be promising against brown spot disease of tobacco in the field.

In vivo evaluation of chemical fungicides showed that Baycor, Bayleton, tricyclazole, difenoconazole and propiconazole were found promising against brown spot of FCV tobacco (Nagarajan and Shenoi, 1998). Shafik and Taha (1984) found that Dithane M-45 when sprayed @ 2.0 g / l water proved effective in controlling the disease.

Dam *et al.* (2010c) indicated that two sprays, one at disease appearance and another after 15 days with Propiconazole @ 0.1% or Mancozeb @ 0.2% were promising in the management of brown spot disease.

Biological management

Dam *et al.* (2010b) have observed that both *Trichoderma viride* and *Pseudomonas aeruginosa* restricted the growth of *Alternaria alternata in vitro* though in the former, the inhibitory effect was more. It was observed that soil application + two foliar sprays, one at disease appearance and another after 15 days with *Trichoderma* + *Pseudomonas* + *Azotobacter* (B:C ratio 1:1.79) was at par with two foliar sprays with propiconazole (B:C ratio 1: 1.74) in reducing brown spot disease of *Motihari* tobacco. Pande (1985) reported that three isolates of *Trichoderma viride* retarded growth of *Alternaria alternata*.

4. *Frog eye leaf spot*

Generally this disease is seen 4-5 weeks after germination and 30 days after transplanting and on the harvested crop. The fungus causing frog eye of tobacco is generally called *Cercospora nicotianae* Ell. and Ev.

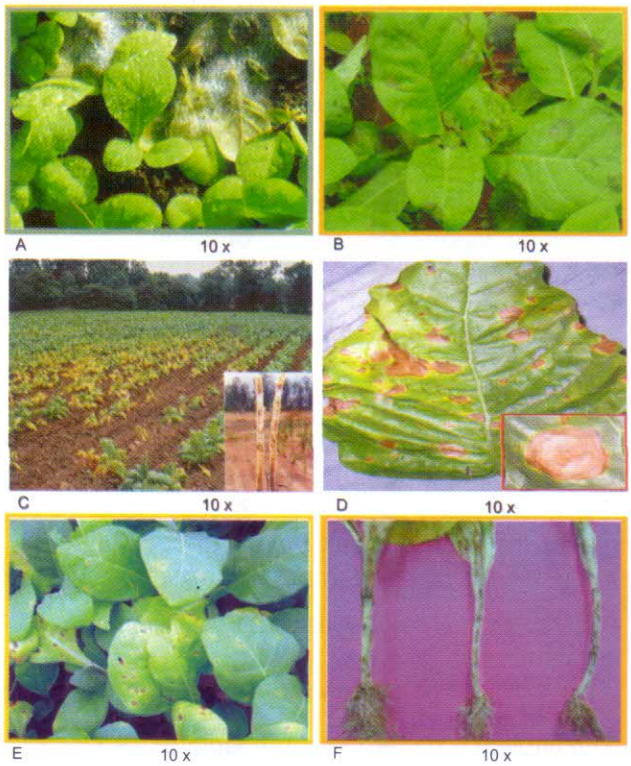

Fig. 1 : Symptoms of fungal diseases, A. Damping off, B. Leaf blight, C. Black shank, D. Brown spot, E. Frog eye leaf spot, F. Sore shin

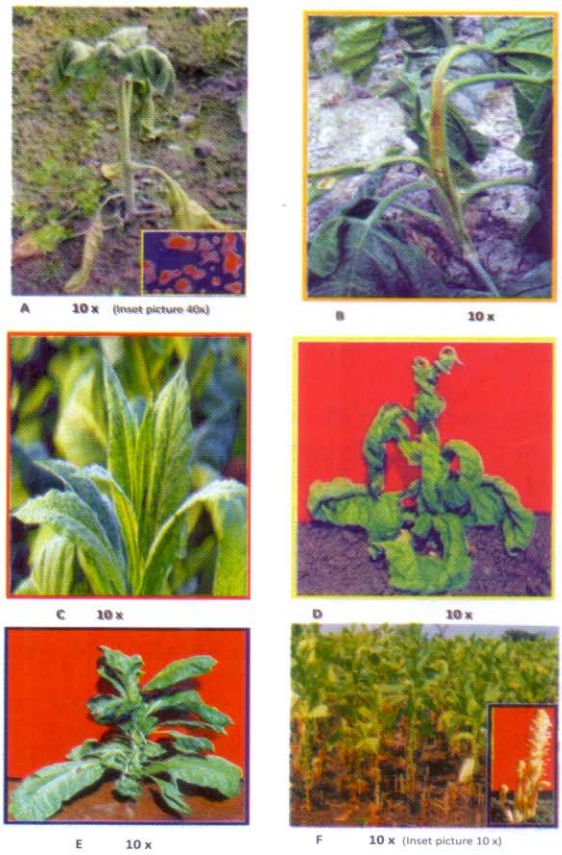

Fig. 2 : Symptoms of bacterial and viral diseases, A. Wilt, B. Hollow stalk, C. Tobacco mosaic virus, D. Tobacco leaf curl virus, E. Cucumber mosaic virus; F. Symptoms of *Orobanche* affected tobacco plants.

Symptoms

Brown, round spots resembling frog-eye form appear on the lower leaves of the seedlings. Spots appear first on basal leaves and gradually spread to upper leaves. Spots are small, circular, 5-8 cm diameter in size, brown or tan with dingy gray centers. Under hot, dry weather frog eye lesions may be pinpoint in size and would not be recognized. The pathogen is regarded as weak parasite which normally attacks only physiologically declining tissue (Fig. 1E).

Favourable conditions and spread of the disease

Frequent watering and wet weather leading to high humidity and temperature around 27^{o}C are favourable for the development of the disease.

Disease management
Cultural management

a. Use recommended fertilizer dose only.
b. Avoid excess nitrogen and similarly do not reduce potash fertilizer dose.
c. Collect and destroy the crop debris after harvest.
d. Always transplant healthy seedlings or remove the spotted basal leaves from seedling before transplanting.
e. Number of watering per day may be regulated depending on the need and weather parameters, so as to avoid excess humidity / dampness on the nursery beds.

Chemical management

In vivo evaluation of chemical fungicides showed that carbendazim, propiconazole, kresoxim methyl, pyraclostrobin + metiram and azoxystrobin were found promising against frog eye spot of FCV tobacco (Anon., 2013).

Spraying carbendazim 50% W P @ 5 g in 10 liters of water on first appearance of the disease. If necessary, the spray after 10 days may be repeated.

5. Sore shin

Sore shin is considered to be a minor disease, though in certain individual fields damage caused is fairly high. The sore shin fungus *Rhizoctonia solani* attacks many plant species of economic importance and can remain as a saprophyte on decaying plants or as sclerotia in soil. It appears both in tobacco nursery as well as in the field.

Symptoms

A seedling stem cancer, known as sore shin, is common and destructive in tobacco. Sore shin lesions appear as reddish-brown, sunken cankers that range from narrow to completely girdling the stem near the soil line. Injury is more common during warm weather. As soil temperature rises later in the season, affected plants may show partial recovery due to new root growth. On transplants, the dark brown discoloration of the stalk at or near the soil line extending upward for several centimeters is a characteristic symptom of sore shin (Fig. 1F).

Mode of entry

Growth of *Rhizoctonia solani* is stimulated by exudates from germinating seedlings and actively growing roots. Once contact has been made *R. solani* can penetrate plant tissue by (i) direct penetration, (ii) forming hyphal aggregations on the roots which cause discoloration and death of root cells with subsequent penetration through this dead tissue, and direct invasion through natural cracks and wounds.

Disease management

Disinfestations of seed-bed soils with steam or methyl bromide before sowing helps to prevent primary infection of sore shin. Terraclor, Chloroneb, Captan, Folpet, Difolatan, Thiram, Plantvax and several other chemicals gave good control, unfortunately were phytotoxic to the tobacco.

In the nursery different chemicals viz., propiconazole, thiophanate methyl, carbendazim, chlorothalonil and copper hydroxide were tested and observed that propiconazole (0.1%), a triazole compound showed superiority in controlling the disease over all other fungicides. The next best chemical identified was carbendazim (Anon., 2010).

B. *Bacterial diseases*
1. *Bacterial wilt*

Bacterial wilt is another important disease of both

Jati and *Motihari* tobacco in *terai* region of West Bengal. Bacterial wilt of tobacco is known to be caused by *Ralstonia solanacearum* and was coined 'Granville wilt' as it was first recognized in FCV tobacco in Granville County during 1880. Except for the prevalence of this disease in *terai* agro-ecological egion of West Bengal, the disease has not been reported from FCV or non-FCV tobacco growing states of India.

Symptoms

The disease initiates right from nursery stage and in adult plants. In the field the first symptom of the disease is drooping of 1-2 leaves during day which may recover during evening. Only half of the affected leaves become flaccid, a characteristic symptom of bacterial wilt of tobacco. On slow progression of the disease, the affected leaves turn light green and may gradually turn yellow, midribs and veins get flaccid and large leaves may droop in an umbrella like fashion (Fig. 2A).

Etiology

Survey of the disease was carried out for *Jati* (*N. tabacum*) and *Motihari* tobacco (*N. rustica*) in villages in and around Dinhata sub-division of Cooch Behar district of West Bengal for the crop season 2003-04 and 2004-05 (Anon., 2004-05; 2005-06) and revealed that the plants aged 35-60 days were found to be highly susceptible to bacterial wilt infection in the field following high temperature (25-30^{o}C) and rainfall. Water logged plots were predisposed to rapid build-up and spread of the disease. Inspite of latent infection in plants, the cause attributed to non-expression of the disease in some plants was due to adult plant resistance. However, impending danger always lay ahead in survival of the pathogen in soil, weed hosts and solanaceous plants (Anon., 2004-05).

The survey revealed that i) increase in temperature from 20-30^{o}C favored wilt development, ii) at lower temp. (< 20^{o} C) disease remain latent and symptom expression did not occur, iii) high soil moisture, temperature in the range of 20-30^{o}C and high relative humidity (> 80) in atmosphere were found to be highly favorable predisposing factors for rapid build- up and spread of the disease (Anon., 2005-06).

Disease management

Cultural management of the disease includes keeping the fields weed free, not to throw the uprooted diseased plants in the field but by burning outside the field and deeply burying it.

The recommended cultural practice from Central Tobacco Research Institute Research Station, Dinhata (W.B.) against bacterial wilt of tobacco is application of lime @ 1 t/ha followed by ploughing and laddering of field and to keep it fallow for 20-30 days. Incorporation of dhaincha as green manure has also found to be beneficial (Anon., 2011).

There is every likelihood that owing to extensive cultivation of bacterial wilt susceptible crops over the last 2-3 decades in *terai* region of West Bengal like potato, brinjal, chilies, tomato, jute and mesta have created enough scope for the progressive build-up and spread of the pathogen in soil and crop debris. This is the reason why the pathogen could establish itself as serious endemic disease of economic importance in *terai* region of West Bengal (Roy *et al.*, 2008).

2. *Hollow stalk*

Hollow stalk of *Motihari* tobacco caused by *Erwinia carotovora* sub sp. *carotovora* (syn. *Pectobacterium carotovorum* sub sp. *carotovorum*) poses serious threat in *terai* region of West Bengal as the disease is endemic in nature. In FCV tobacco (*N. tabacum*) hollow stalk disease has been reported from U.S.A, Canada and China affecting mainly stem (black leg) and cured leaves in stores. From India hollow stalk has been reported from *N. rustica* type of tobacco only and to a lesser extent in *Jati* tobacco of *N. tabacum* type from *terai* region of West Bengal (Anon., 2004-05; 2005- 06; Roy *et al.*, 2008).

Symptoms

Hollow stalk in the field usually appears first at topping and suckering time. The disease may appear at any time following stem injury but it is commonly observed after 35-40 days during topping operations. Soon after infection a rapid browning of the pith develops followed by soft rot and eventual collapse of the tissue. The top leaves wilt and the infection spreads downwards. Hollow stalk phase of the disease characterized by hollowness of the stem following soft rot which has been reported from FCV (*N. tabacum*) tobacco (Lucas, 1975) as well as from *N. rustica* tobacco (*Motihari* tobacco) and to a very limited extent in *Jati* tobacco (*N. tabacum*) from terai region of West Bengal (Fig.

2B). Black leg phase of the disease characterized by formation of blacks stripes or bands girdling the stalk and cured leaves have been reported from USA, Canada (Lucas, 1975) and China (Xia and Mo, 2007) but not from India.

Losses

Hollow stalk of *Motihari* tobacco poses serious threat in *terai* region of West Bengal as the disease is endemic in nature and the losses on an average caused to the crop range from 0.5 - 30 %. In case of higher latent infection in plants, the entire crop can be wiped out in the event of high rainfall and water logging of soils (Anon., 2004-05; 2005-06).

Screening for disease resistance

Out of a total of 185, *N. rustica* germplasm accessions screened for resistance to hollow stalk disease, only two accessions viz. White Pathar and Bengthuli exhibited resistant disease reaction (< or upto 2 cm linear soft rot of pith on artificial inoculation) (Anon., 2010).

Disease management

Utmost care should be exercised during field operations not to use unsterilized sickle /knife lest the disease is spread in healthy plants.
Field operations like desuckering and topping should be avoided during damp and cloudy weather.

Effective prophylactic management of the disease has been recommended as slurry/paste spot application of Bordeaux mixture or Copper oxychloride at topped end and desuckered points of leaf (Anon., 2005-06).

After the onset of disease, the most effective management strategy lies in providing deep incision with sterilized knife at the stem base or desuckered points of leaf followed by paste application of Bordeaux mixture or Copper oxychloride.

C. Viral diseases

1. Tobacco Mosaic Virus (TMV)

Tobacco mosaic virus (TMV) is a single-stranded, positive-sense, rod shaped RNA virus that is worldwide in distribution and is found in all countries where tobacco is grown. TMV reduces cured tobacco yield, quality, and average price. Because of its adverse effects on tobacco and the high value of the crop, TMV is an economically important disease. Since then TMV has been acknowledged as preferred didactic and symbolic model to illuminate the essential features that define virus. Today, TMV is used as a tool to study host –pathogen interactions and cellular trafficking, and as technology to express valuable pharmaceutical proteins intobacco (Scholthof, 2004).

Beijernick (1898) contended that the filterable agent of tobacco mosaic disease was neither bacterium nor any corpuscular body, but rather that was 'contagium vivum fluidum'. The path-breaking work on tobacco mosaic virus was that it could pass through a filter capable of retaining bacteria (Mayer, 1886; Ivanowski, 1892; Zaitlin, 1998). TMV was the first to be chemically purified (Stanley, 1935; Bawden *et al.*, 1936), to be detected in centrifuge and in electrophoresis apparatus (Eriksson-Quensel and Svedberg, 1936) and to be visualized in an electron microscope (Kausche *et al.*, 1939). The TMV coat protein (CP) was the first virus protein to be sequenced (Anderer *et al.*, 1960; Tsugita *et al.*, 1960) and TMV's particle structure was the first to be elucidated in atomic detail (Bloomer *et al.*, 1978; Namba *et al.*, 1989).

Host range

Tobacco mosaic virus has a wide host range, including 199 species from 30 families. However, other *Solanaceous* hosts are the only important sources of inoculum for the tobacco crop (Shew and Lucas, 1991).

Symptoms

Characteristic symptoms include an irregular pattern of dark green and light green leaf areas intermingled, stunted plant growth, leaf malformation, and mosaic burn. This mosaic pattern is the result of intermingled yellow-green mottling on the foliage of the tobacco plant. Young leaves of infected plants are often malformedand may show leaf puckering, or wrinkling of the leaf tissue. Nearly mature leaves that are infected may show "mosaic burn" (Fig. 2C). Mosaic burn is characterized by large, irregular, burned or necrotic areas on the foliage that can cause extensive damage to the tobacco crop (Lucas, 1975).

Mode of transmission

Tobacco mosaic virus is sap transmissible and is

one of the most infectious plant viruses. Primary sources of infection may include perennial weeds and infected crop debris in the soil. Primary infections are usually only responsible for a small proportion of infected plants in a field. Gooding (1969) reported that only about 10% of TMV infection in North Carolina is the result of primary infection by crop debris in the soil. Therefore, secondary spread of the virus accounts for most TMV infections. The virus is mainly spread by contact. Secondary infections may occur when a worker's hands, clothing, or equipment, previously in contact with an infected plant, comes in contact with a healthy plant. Cultivation practices such as hoeing, topping, spraying pesticides and insecticides, and other field operations can also spread the disease (Lucas, 1975).

Another source of new infection is air-dried tobacco. Workers who smoke cigarettes, or who use chewing tobacco or snuff containing air-cured tobacco, may introduce the virus to the plants. This is especially common when the worker is performing plant bed operations. Leaf symptoms do not usually have time to develop before transplanting, so mosaic is rarely seen in the plant beds. However, if TMV is present in the plant beds, then it could easily be spread at transplanting time when the infected plants come in contact with workers and equipment.

Environmental factors

Plant age, amount of inoculum and growth conditions in general determine whether symptom expression is acute or chronic. Usually the time required for symptom appearance is shortened by increases in temperature and light intensity. Above 38-40^{o}C infection is inhibited and above 27^{o}C or below 10^{o}C symptoms disappear.

Disease management
Preventative and Eradicative

According to Lucas (1975), "The most efficient way to control mosaic is to keep the crop TMV-free". Some preventative measures include use only resistant varieties, if available; strictly follow the sanitary measures; rouging of diseased plants early in the season; take up inter-culture operations in infected fields at the end of the day and disinfect the implements before entering healthy fields; workers should wash their hands with soap water before and after entering infected fields; workers should avoid smoking and use of other tobacco products.

Prophylactic sprays of 0.5% skimmed milk on 30th, 40th and 50th DAP (Hare and Lucas, 1959) prevent spread of the disease.

Foliar spray of dodine and glyodin, have found to reduce the amount of damage caused by TMV when applied in the field (Chow and Rodgers, 1973).

Host resistance

Host resistance is an effective means of controlling most pathogens. Apple *et al.* (1963) found that host resistance derived from *N. glutinosa* reduced losses in yield and value to TMV more than did milk treatment of seedlings.

Sources of resistance

The first resistance to TMV incorporated into tobacco came from the Colombian variety '*Ambalema*' (Nolla and Roque, 1933). Once resistance was incorporated into the tobacco genome, the plants were of poor agronomic quality. Holmes (1938) used inter-specific hybridization to incorporate resistance to TMV from *Nicotiana glutinosa* into the Turkish tobacco variety Samsun.

Resistance derived from *Nicotiana glutinosa* ('N' gene) was successfully incorporated into burley tobacco. However, when the 'N' gene was incorporated into flue-cured tobacco, Chaplin *et al.* (1961) reported reductions in cured tobacco yield and value, and Chaplin and Mann (1978) concluded that the N factor may be inherently difficult to disassociate from the adverse yield and quality characteristics in flue-cured tobacco.

Reddy and Nagarajan (1981) observed that among the 268 collections of *N. tabacum* and *N. rustica* screened 8 FCV, 10 Burley, 10 Air cured, 3 *rustica*, 2 each of hookah and cigar and 1 each of natu and snuff types were found resistant, the resistance being *glutinosa* type, showing typical hypersensitive local lesion reaction.

Sastri *et al.* (1981) found that three Tobacco mosaic Virus (*Nicotiana* 1) resistant flue-cured tobacco variety viz. TMVRR-1, 2 and 3 were superior over their susceptible counter parts viz. Virginia gold, CTRI Spl. and Kanakaprabha under heavy infection condition. Shenoi *et al.* (1992) screened 216 *Nicotiana* germplasm and found that 36 entries

were resistant to TMV showing hypersensitive *glutinosa* reaction, and one as tolerant to TMV.

Management with botanicals

Prophylactic sprays of plant extract like *Basella* or *Bougainvillea* or neem @ 1% (Nagarajan and Murthy, 1975) prevent spread of the TMV disease. Nagarajan and Murthy (1977) screened 27 leaf extract of several plants and found that among them *Acacia Arabica*, *Boerhaavia diffusa*, *Lawsonia inermis*, *Nerium odorum*, *Peltophorum ferrugenium*, *Pithecolobium duice* and *Prosopis specigera* where the TMV inhibition was more than 80 per cent.

Shamarao *et al.* (2011) found that application of Viroson 2% (27.7% disease incidence) followed by Bougainvillea leaf extract 5% (30.2% incidence) and neem 1500 ppm (31.8%) reduced the TMV incidence. However, higher plant height, leaf length and leaf width were recorded in Viroson, neem 1500 ppm and cow urine application indicating their role in triggering host defense and plant growth promotion.

2. Tobacco Leaf Curl Virus (TLCV)

This disease is caused by a virus belonging to 'Gemini' virus group. The virus is transmitted by insect-vector whitefly, *Bemisia tabaci* Gennadius. It is neither seed borne nor transmitted mechanically. Intensity and spread of the disease depend on plant age at the time of infection and build-up of whiteflies. Whiteflies are more abundant and active in relatively dry season and as a result, leaf curl is more prevalent during dry weather (Lucas, 1975). Whereas high rainfall, more number of rainy days and higher relative humidity, accompanied by less bright sunshine hours favoured low leaf curl incidence (Monga and Tripathi, 1988). The virus remains persistent in the whitefly vector. In severe infection, losses vary from 60-70% as infected leaves do not cure well. In mild type of infection, the yield loss is very marginal.

Valand and Muniyappa (1992) reported the highest incidence of disease was observed in Andhra Pradesh (77%) followed by Gujarat (59%), Karnataka (17%), Bihar (11.6%) and West Bengal (5.4%).

Symptoms

Diseased leaves show vein clearing, puckering of leaves, downward curling of leaf margins; leaves become brittle; thickening of veins, ear-like outgrowths on the under surface of leaves resulting in stunted growth of the plant (Fig. 2D).

Mode of entry

Infective whiteflies insert the virus into phloem tissues by means of the stylets while feeding on the leaves. The incubation period varies from 12 to 33 days depending on temperature, plant age and vigor. The disease is seldom seen in the plant bed, but usually appears 2 to 3 weeks after transplanting.

Spread of the pathogen

A common vector of TLCV is the whitefly, known generally as *Bemisia tabaci* Gennadius, a member of the family Aleyrodidae. The virus persisted in the vector for at least 6 days. Single viruliferous whiteflies were found to be efficient vectors.

Disease management

As TLCV is a virus, no direct method for control is as yet available. The incidence of TLCV can be decreased by vector control. The application of chemicals has been reported to be effective in the management of *Bemisia tabaci*.

1.Leaf curl infected seedlings should be discarded while planting.

2.Diseased tobacco plants should be removed and destroyed within one month after planting if the infected plants are less than 2%.

3.Alternate weed hosts for whitefly should be removed and destroyed, in and around tobacco fields.

4.Soon after harvesting the tobacco crop should be destroyed to prevent overwintering.

5.Crops like brinjal and sunflower should not be grown in the vicinity of tobacco fields.

6.12 yellow sticky traps (castor oil coated) per hectare should be installed to monitor the whitefly population. If 100 whiteflies stick to the trap the following insecticide, spray schedule has to be adopted.

Imidacloprid 200 SL @ 2.5 ml or thiomethoxam @ 2 g/10 litres of water ten days before pulling in the

nursery, 10 days after transplanting in the field 2-3 times at an interval of 10 days.

3. Cucumber Mosaic Virus (CMV)

The cucumber mosaic virus has one of the broadest host ranges. The virus is distributed worldwide and the symptoms it causes are easily mistaken for tobacco mosaic.

Symptoms

Typical mottling and mosaic patterns appear, sometimes accompanied by stunting and narrowing and distortion of the leaves (Fig. 2E). Severe strains may cause interveinal discoloration and the oak-leaf pattern of necrosis on the lower leaves. 'Mosaic-burn' or sun-scald frequently appears on the upper leaves of infected plants. Mild strains cause only a faint mottling of the leaves.

Life cycle

The cucumber mosaic virus overwinters in perennial weeds and may be transmitted to healthy plants by aphid vectors or by mechanical means. The cucumber mosaic virus cannot withstand drying or persist in the soil. It also is more difficult than tobacco mosaic to transmit mechanically. Thus, cucumber mosaic tends to progress more slowly than tobacco mosaic in a field.

Mode of entry

CMV is introduced into the leaf through wounds, principally those made by aphid. Bradley (1968) showed that the mid veins and secondary veins were excellent sources of CMV and that aphids probing infected tissue for less than a minute became highly viruliferous.

Basic Studies – Mechanism of resistance/ Biotechnological approach of resistance

Couzzo *et al.* (1988) demonstrated coat protein (CP) mediated protection in CMV. Quemada *et al.* (1991) engineered the coat protein (CP) gene from cucumber mosaic virus (CNV) strain C and transferred them into genome of tobacco (*N. tabacum* 'Xanthi'). They infected transgenic tobacco plants with CMV strain C of chi of sub group I and strain WL of subgroup II, transmitted mechanically or by aphids. They found significant degree of protection when challenged with CMV strains of either sub-group.

Rizos *et al.* (1996) reported that transgenic plants expressing coat protein (CP) of an Australian isolate of cucumber mosaic virus (CMV) were resistant to infection with the homologous and two heterologous CMV strains. The level of resistance observed in CP - expressing plants was related to the virulence of challenging CMV isolate and not to the similarity between the CP expressed by transgenic plant and CP of the challenging virus.

A mutant of the cucumber mosaic virus subgroup I A strain Fny (Fny-CMV) lacking the gene encoding the 2b protein (Fny- CMV delta2b) induced symptomless systemic infection in tobacco. Both the accumulation of Fny-CMV delta2b in inoculated tissue and systemic movement of the virus appear to proceed compared towild type Fny-CMV (Soards *et al.*, 2002).

Disease management

·The virus is readily transferred by aphids and survives on a wide variety of plants. Varietal resistance is the primary management tool, and eliminating weeds and infected perennial ornamentals that may harbor the virus is critical.

·Viral diseases cannot be controlled once the plant is infected. Therefore, every effort should be made to prevent introduction of viral diseases into the field. Sanitation is the primary means of controlling viral diseases. Infected plants should be removed immediately to prevent spread of the pathogens. Perennial weeds, which may serve as alternate hosts, should be controlled in and adjacent to the field.

Spraying of imidacloprid 200 SL @ 2.5 ml or thiomethoxam @ 2 g/10 litres of water ten days before uprooting of seedlings in the nursery and 10 days after transplanting in the field 2-3 times at an interval of 10 days are to be followed.

D. Phenerogamic Parasite Orobanche

Orobanche is popularly known as 'broomrape' in English and 'bodu' or 'malle' in Andhra Pradesh. *Orobanche* belongs to family Orobanchaceae and order Scrophulariales. This is a complete root para-

site on many solanaceous plants, including tobacco.

Symptoms

It is a flowering parasitic occurring on roots of tobacco plants. The shoots emerge in clusters and their basal portion is attached to tobacco roots through which it draws nourishment and depletes the host plant. Affected plants become stunted. Leaves turn pale and wilt. Initially leaf tips droop and as the attack intensifies all the leaves wilt with characteristic ribbing of midribs (Fig. 2F). High soil moisture due to irrigation or rain after planting, low soil temperature during winter months encourage heavy incidence of *Orobanche*. It is more severe in black cotton soils than in light soils. Yield losses due to early infection were estimated to be around 30%. Leaf quality is also greatly affected.

Etiology

At least 7 species of *Orobanche* are known to parasitize the genus *Nicotiana* but *Orobanche cernua* Loefl. is the most prevalent in India.

Life cycle

The parasite perennates through seed. Seeds of *Orobanche* remain viable in the soil more than 10 years in absence of the host. Once tobacco is planted, within 2 weeks the root exudates induce germination of *Orobanche* seed and produces a shallow disc or cup-like appressorium which surrounds the host root, penetrates it with a mass of undifferentiated, polymorphic cells that extend into the xylem of the host root and absorb nutrients and water from it.

Disease Management

Sustainable management of the parasite can only be achieved by reducing the soil seed bank.

Cultural and preventive measures

a)Deep summer ploughing of the sick field with disk plough 2-3 times exposes the *Orobanche* seed to the hot sun and results in desiccation. The seed on the upper layers of the soil are likely to be buried deep in the soil, beyond the reach of tobacco roots.

b)Thick sowing of any of the trap crops like Jowar, Gingelly, Greengram, Black gram, Pillipesara (*Phaseolus trilobus*) etc. in *Kharif* induce germination of *Orobanche* seed upto 30%. The germinated seeds die as they cannot infest the trap crops.

c)Avoid growing of brinjal, tomato, bhendi and other Solanaceous crops in the sick fields.

d)Application of any of the copper fungicides @ 0.2% at the time of planting delays emergence of *Orobanche* by few days and also protects the seedlings from black shank.

e)Periodical removal of emerged *Orobanche* spikes either manually or by using 'spear' an instrument to cut the spikes at soil level, before flowering or seed setting helps in reducing the inoculum in the soil. Following this method meticulously for 3 to 4 years reduces *Orobanche* incidence greatly (Pal and Gopalachari, 1957; Krishnamurthy and Krishnan, 1967; Krishnamurthy *et al.*, 1991a).

f)Late planting is better to reduce *Orobanche* infestation in Tobacco (Okazova, 1975).

Chemical management

Krishnamurthy *et al.* (1976) have observed that swabbing *Orobanche* shoots with kerosene oil gave maximum mortality (84.8%) and quick knock-down effect. Spray/drenching with 0.2% allyl alcohol gave moderate kill (47.6%) followed by 0.125% of I.C. 21 (18.8%) and 0.125%, DNOC (18.0%). Krishnamurthy *et al.* (1991b) tested Eucalyptus, Pongamia, Rice Bran, Soybean and Tobacco seed oils for the control of broomrape (*Orobanche cernua*) in tobacco. The oil was applied to young, unflowered broomrape shoots at 1-5 drops/shoot with a dropper. All the oils effectively killed the parasitic shoot, their optimum doses being 1 drop/shoot for Eucalyptus, Pongamia, Soybean and Tobacco seed oils and 2 drops for Rice bran oil.

In Italy, Sandri *et al.* (1998) observed that Glyphosate @ 1 lit/ha gave good control of *Orobanche ramosa* in Burley tobacco. In India, Dhanapal *et al.* (1998) have reported that Glyphosate at 500 g ai/ ha at 60 days after transplanting (DAT) and Imazaquin at 10 g ai/ha at 30 DAT reduced the spikes by 75 to 80%, respectively.

Biological management

Orobanche cernua is a serious parasite in most of the tobacco growing regions in India causing quantitative and qualitative losses. *Sclerotium rolfsii* was reported to cause a serious disease in *Orobanche* parasitizing tobacco, effecting the seed production of the parasite. However, since this is a polyphagous pathogen and under high moisture conditions, it is likely to attack the host crop tobacco, the patho-

gen is still under investigation to be considered as biocontrol agent for *Orobanche* (Raju *et al.*, 1995).

Among other fungal pathogens infecting *Orobanche* spp., *Fusarium* spp. seems to be highly widespread. Many scientists reported its association with the parasite in different crops (Ramaiah, 1987; Mazheri *et al.*, 1991; Bedi and Donchev, 1991 and 1995; Linke *et al.*, 1992 and Parker and Riches, 1993). Though other fungi like *Alternaria*, *Urocladium* etc. have also been observed to be associated with diseased *Orobanche* spikes, *Fusarium* spp., especially *oxysporum* f. sp. *orthoceros* and *lateriticum* appear to be highly potential and also widespread. It is interesting to note that *Fusarium* controlled 50 to 90 per cent of *Orobanche* in different countries. The fungus applied as conidiophores was the most virulent and *Orobanche* was most susceptible between germination and tubercle formation (Bozoukov and Kouzmanova, 1994).

Other fungi like *Alternaria*, *Trichoderma*, *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Cladosporium*, *Trichothecium* etc. have also been reported to be associated with the diseased *Orobanche* spikes but their role in the biological control has not been established (Abdel-Kader *et al.*, 1996 and Al-Menoufi, 1994).

Okazova (1973), Klein and Kroschel (2002) have reported that the larvae of *Phytomyza orobanchia* mine in *Orobanche* shoots and capsules. As a consequence, a natural reduction of *Orobanche* seed production by 30 to almost 80% has been reported from different countries. To strengthen the natural population and its impact, inundative releases of *P. orobanchia* adults at the beginning of *Orobanche* emergence have to be undertaken.

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