ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.8, pp.001-011, September, 2010

REVIEW ARTICLE

CURRENT STATUS OF ANGULAR LEAF SPOT (*Pseudomonas syringae* pv. *lachrymans*) OF CUCUMBER: A REVIEW

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ARTICLE INFO

Article History:
Received 19th June, 2010
Received in revised form
13th July, 2010
Accepted 19th August, 2010
Published online 4th September, 2010

Key words:
Angular leaf spot, cucumber, *Pseudomonas syringae* pv. *lachrymans*

ABSTRACT

Angular leaf spot *(Pseudomonas syringae* pv. *lachrymans)* is more or less prevalent in almost every cucumber growing area of the world causing heavy losses not only to cucumber but to other cucurbits as well. Under natural conditions, the disease initially appears as minute water-soaked spots on leaves, which became and turn light tan in colour. With the passage of time, spots turn necrotic and many of these slough-off giving leaves a tattered appearance. The spots on infected fruits lead to rot or misshapening of fruit in case of severe infection. Both naturally infected as well as the artificially inoculated seed can harbour the pathogen beyond two sowing seasons. Pathogen survives on diseased crop debris buried or kept on soil surface from harvesting to next sowing time. The disease is controlled by hot water treatment, chemical seed treatment, foliar sprays and use of systemic resistance inducers.

INTRODUCTION

Cucumber is grown throughout the world over an area of 1563 thousand hectares with a total production of 26582 thousand metric tones. From nutritional point of view, cucumbers stand for low calorific value but possess appreciable quantities of vitamin B and C. Cucumber fruits are having cooling effects, prevent constipation and are useful to jaundice patients, besides their seeds being used in many *ayurvedic* preparations (Ram, 2002). Yield and quality of cucumber is affected by a number of fungal, bacterial and viral diseases. Of these, angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* is one of the serious diseases of the crop, which can inflict upto 37 and 40 per cent reduction in fruit number and fruit weight, respectively besides rendering some fruits as unmarketable culls (Pohronezny *et al*., 1977). Under favourable climatic conditions, the disease becomes extremely destructive inflicting severe damages on cucumber and other cucurbits thereby causing economic loss to farmers. Annual economic loss of 0.5 million dollars in production of cucumber crop due to this disease, alone in Wiscosin state of USA has been reported (Kennedy and Alcorn, 1980). The disease poses threat not only to cucumber but also to a number of cultivated and wild cucurbits throughout the world. In this article, an attempt has been made to review the relevant available literature concerning angular leaf spot of cucumber.

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Occurrence and the severity of the disease

The first authentic report of angular leaf spot of cucumber along with accurate description of the pathogen was given by Smith and Bryan in 1915 from USA and since then the disease is considered as one of the most serious diseases of cucumbers in United States. Subsequently, occurrence of the disease was reported from Russia, Japan, England, Israel, and Iran. The disease has also been reported to prevail in China, Turkey, Australia, Argentina, Brazil and most of the European and African countries, and has therefore, attained the status of worldwide occurrence (Bradbury, 1986). Due to the versatile nature of the pathogen, the disease has been reported as a serious problem of cucumbers from California with somewhat warmer climate (Stout, 1952) and from Denmark, which is quite cooler region (Hellmers, 1950). Ark and Gardner (1956) have also reported a severe damage of cucumbers in some coastal areas of California due to this disease. It is also reported to be the heaviest and most widely spread disease of pickling cucumbers in Connecticut valley, USA (Boyd, 1942).

Different reports of severity and damages caused by the disease reveal its importance as a serious constraint in cucumber cultivation. The disease has been reported to cause a yield reduction of upto 50 per cent in Moscow province of Russia (Gorlenko and Voronkevich, 1946). It had attained such a severe status in cucumber growing districts of Japan that 6240 hectares were affected in 1980,

counting for about a half of the total cucumber planted area (Watanabe and Ohuchi, 1983). Mabbett and Phelps (1984) reported that angular leaf spot is a perennial problem and a serious constraint in cucumber production in Trinidad, West Indies which can cause secondary infection of over 80 per cent of leaves by the tenth day of first infection during wet conditions. Cucumber seedlings raised from infected seeds in Egypt are reported to have a disease incidence of 98 per cent (El-Sadek *et al*., 1992). Mohamed *et al*. (2000) reported from Egypt that infection of *P. syringae* pv. *lachrymans* caused a reduction of 4.4 to 30.05 per cent in dry weight, 8.2 to 35.4 per cent in water content, 7.0 to 13.9 per cent in shoot length and 16.5 to 25.2 per cent in root length of different cultivars of cucumbers.

In India, angular leaf spot of cucumber was first reported by Papdiwal and Deshpande (1978) from Aurangabad, Maharashtra. Later on, Jindal and Bhardwaj (1991) and Jindal (1994) reported angular leaf spot on Persian melon and cucumber, respectively from Himachal Pradesh, India, where the disease had caused a severe epidemic on cucumber during July 1992. The disease has been reported to occur in all cucumber growing areas of Kashmir valley with incidence and intensity ranging between 23.35 to 74.45 % and 10.50 to 26.02 %, respectively (Bhat *et al*., 2007).

Symptoms of the disease

The symptoms of angular leaf spot of cucumber are evident on cotyledons, leaves and fruits, and have been studied extensively by various workers.

Symptoms on Cotyledons

Symptoms on cotyledons of seedlings growing from infected seeds were extensively studied by Wiles and Walker (1951) who reported that the lesions on cotyledons were variable in size and shape often visible on their outer surface as soon as cotyledons emerge from seed coat or may appear along margins near the growing point and on the inner surfaces of the cotyledons. The cotyledonary lesions may turn light tan to brown in colour and also show bacterial exudates during humid periods. Keppler and Novacky (1986) reported that the first visible symptom on cucumber seedlings was tissue collapse which began at the edges of the cotyledon. On the other hand, Wyszogrodzka *et al.* (1987) reported that the symptoms on cotyledons appeared as brown necrotic tissue expanding into a concentric zone, 1 to 2 mm wide, of conspicuous chlorosis.

Symptoms on Leaves

Spots on leaves are water soaked, which become angular being delimited by veins, turn grey to tan in colour forming an exudate on lower surface of the leaf and finally a good many of the spots loosen and fall out (Jindal, 1994; Verma and Sharma, 1999). However, Hellmers (1950) reported that the angular, pale fawn to flaxen, shriveled lesions often 1 to 8 mm in diameter, sometimes merge into large necrotic blotches on a susceptible variety. Van Gundy and Walker (1957a) reported that leaves became severely diseased if these approached maximum size at the time when conditions were favourable for infection, whereas older leaves and young unfolding leaves at growing tips of stems were relatively free from lesions. Saleh and Korobko (1981) reported that freshly isolated strains of *P. syringae* pv. *lachrymans* could induce wilting of cucumber shoot in addition to the formation of irregular necrotic leaf spots, but the wilt inducing ability of the pathogen was lost after its prolonged storage on synthetic media. Different isolates of *P. syringae* pv. *lachrymans* from Egypt are also reported to induce wilting, besides the typical angular leaf spots, within 3 to 6 days of inoculation (Mohamed *et al.,* 2000).

Varied degrees of relative humidity are reported to result in varied symptoms. Typical large lesions are developed at higher levels of relative humidity (90 to 100 %) but only minute lesions at the lower relative humidity. The pathogen is reported to cause only slight marginal blight lesions on cucumber leaves of the plants grown in plastic houses equipped with dehumidifiers for keeping atmospheric humidity below 90 per cen (Watanabe and Ohuchi, 1983),

Symptoms on Fruits

Wiles and Walker (1951) reported that when the pistillate blossoms and very young fruits are attacked by the bacterium, they are generally shed from the vine in a short time. However, fruit infection in the field is most often observed when the fruits have reached about half their normal size and exhibit numerous small, circular, initially water soaked, slightly sunken lesions, which do not increase appreciably in diameter but may coalesce to appear bigger, turning white to tan in centre with age, having curled or fringed appearance of margins. These lesions may be shallow or extend into fruit causing brownish discolouration of the tissues. Bacteria advance throughout the entire length of the fruit resulting in fruit-rot phase of the disease. The authors further reported that the pathogen was present in placental tissue of infected fruits and within the funiculus leading to the seed. Most of these fruit spots have been reported to be formed on flat or concave side of the infected fruit. Secondary soft rot invaders are reported to make their entry into the fruit through cracks developed on disease lesions of a mature fruit and play a role in fruit decay (Verma and Sharma, 1999). Chand and Walker (1964b) reported that when very young cucumber fruits are infected by angular leaf spot disease, they become misshapen often referred to as "Crooks" and are discarded as unmarketable culls. Watanabe and Ohuchi (1983) inoculated *P. syringae* pv. *lachrymans* into cucumber fruit segments by needle pricking method and reported conspicuous ooze exudation on inoculated sites after their incubation in moist chamber. Exudation of amber coloured ooze from the water soaked lesions of Persian melon fruit has also been reported by Jindal and Bhardwaj (1991).

Symptoms on Seeds

No symptoms are apparent on seeds, which are obtained from fruits naturally infected by *P. syringae* pv. *lachrymans* or artificially inoculated by it. However, naturally infected and the artificially inoculated seeds produced seedlings which had characteristic angular leaf spot lesions on their cotyledons (Leben and Sleesman,

1981). Komoto and Kimura (1983) reported that seeds naturally infected with the angular leaf spot bacterium shrinked partially upon drying and the space thus created contained tissues infected with the bacteria.

Symptoms on Stem

Wiles and Walker (1951) in an attempt to produce systemic infection, inoculated main cucumber stems by *P. syringae* pv. *lachrymans* through needle punctures and observed formation of small local cankers near inoculated sites. Pohronezny *et al.* (1977) inoculated *P. syringae* pv. *lachrymans* into leaf mid vein by small sterilized needle and observed water soaking and white to amber exudates on the surface of leaf petioles and the internode just below the diseased petiole. They further reported that although systemic movement of bacterium to other internodes was also observed but no external symptoms were observed on them.

Pathogen and its characterization

The morphological, cultural and biochemical characters of the bacterium associated with angular leaf spot of cucurbits have been investigated by workers of different countries. Smith and Bryan (1915) were first to study the angular leaf spot pathogen and named it as *Bacterium lachrymans* Smith and Bryan 1915. Afterwards different synonyms of the same organism were used by various workers from time to time, viz; *Pseudomonas lachrymans* (Smith and Bryan) Casner 1918, *Phytomonas lachrymans* (Smith and Bryan) Bergey *et al*. 1923; *Chlorobacter lachrymans* (Smith and Bryan) Patel and Kulkarni 1951; and *Pseudomonas lachrymans* f.sp. *cucumis* Gorlenko 1961. According to the international code of nomenclature of bacteria and international standards for naming pathovars of phytopathogenic bacteria, this organism is named as *Pseudomonas syringae* vanHall, and this species includes a large number of pathovars that cause diseases on many plants and are not distinguishable with certainty by phenotypic characterization, without knowledge of their hosts (Dye *et al*., 1980). For example, Ohuchi *et al*. (1980) reported that bacteriological properties of *Pseudomonas lachrymans* were so similar to those of *P. tabaci* that they were indistinguishable unless their pathogenicity and symptoms were taken into consideration. *P. syringae* group of phytopathogens consists of a number of pathovars, including *pv.lachrymans*, which in past had been given species rank but these species were so poorly characterized that they could not be included in the 1980 list of Approved Bacterial Names (Skermann *et al.*, 1980). As such a pathovar system of nomenclature was devised to conserve the former names of species, which were of significance to plant pathologists (Braun-Kiewnick and Sands, 2001).

Therefore, in the current pattern of nomenclature, the pathogen associated with angular leaf spot of cucurbits is designated as *Pseudomonas syringae pv. lachrymans* (Smith and Bryan) Young, *et al.* 1978, which shares most of its characters with the species barring the host specificity and a few bacteriological characters distinguishing it from other pathovars of the species (Bradbury, 1986).

Cultural characters of the pathogen

Smith (1946) reported that *Pseudomonas lachrymans,* isolated from cucumber or muskmelon, formed white, slightly raised, transparent, smooth surfaced and circular colonies with irregular edges on beef-extract agar producing abundant green fluorescent pigment but the colonies were slightly raised, filiform, white and butyrous on potato dextrose agar. Palleroni (1984) reported that King's medium B is frequently used for direct isolation of fluorescent pseudomonads and their colonies can be identified on the plates by characteristic diffusible pigments. Saprophytic pseudomonads are reported to produce more fluorescent pigment on King's medium B than the pathovars of *Pseudomonas syringae,* which fluoresce blue by visualizing the colonies under UV light (Braun-Kiewnick and Sands, 2001). *Pseudomonas syringae pv. lachrymans* and other pathovars of this species are reported to form levan type colonies on nutrient sucrose agar within three days, which constitutes as a distinguishing character of the species (Bradbury, 1986).

Morphological characters

Smith (1946) studied the morphological characters of *Pseudomonas lachrymans* and reported that the bacterium was Gram-negative, rod shaped with rounded ends, non-acid fast, motile with one to three polar flagella. Kagiwata (1990) studied the morphological characters of several isolates of *P. syringae pv. lachrymans* isolated from all over Japan and reported that all were Gram-negative, aerobic, non-sporing, straight rods and motile with one to five polar flagella. Bradbury (1986) in his detailed account of genus *Pseudomonas*, its species and pathovars reported that pseudomonads are Gram-negative, aerobic rods, straight or curved and range 0.5-1.0 × 1.5-4.0 μm in size.

Bio-chemical Characters

Smith (1946) reported that *P. lachrymans* was negative for nitrate reduction, production of indole and hydrogen sulphide, and starch hydrolysis but was positive for ammonia production. He further reported that the bacterium utilized arabinose, xylose, sucrose, dextrose, levulose, galactose, mannose and sorbitol but not rhamnose, maltose, lactose, trehalose, raffinose, melizitose, melibiose, cellobiose, starch, inulin, dextrin, glycogen, inositol, glycerol, dulcitol, erythritol, salicin, esculin and arbutin. In his description of genus *Pseudomonas*, Burkholder and Starr (1948) reported that most of its members were aerobes or facultative anaerobes, turned milk alkaline, did not hydrolyze starch, did not produce indole and hydrogen sulphide, utilized monosaccharides but not as a rule the higher carbohydrates with the exception of sucrose, and most species could utilize asparagine as the sole carbon and nitrogen source. The authors further pointed out that utilization of sucrose and gelatin liquefaction were two specific characters on the basis of which members of the genus could be differentiated.

Lelliot *et al.* (1966) suggested that pathogenic and non-pathogenic fluorescent pseudomonads can be divided into five groups on the basis of Levan production (L), oxidase

reaction (O), rotting of potato (P), arginine dihydrolase activity (A) and tobacco hypersensitivity test (T), collectively known as LOPAT tests, which provided a useful and simplified operational key for determinative purposes. The authors further reported that *P. lachrymans* was positive for Levan production, tobacco hypersensitivity and sucrose utilization but negative for oxidase, potato rot, arginine dihydrolase and nitrate reduction with different isolates giving varied results in case of tyrosinase activity and aesculin utilization. Tobacco hypersensitivity reaction of *P. syringae pv. lachrymans* and some other related bacteria is reported to be distinctly manifested when 1 to 3 day old bacterial cultures are used but this ability decreased with age, and 10 to 14 days old cultures were not able to evoke the hypersensitive reaction (Gvozdyak *et al.,* 2001). Misaghi and Grogan (1969) emphasized the significance of LOPAT tests in identification of fluorescent pseudomonads after conducting these tests for 26 pathogenic isolates of *P. lachrymans*. The authors reported that all the 26 isolates were positive for tobacco hypersensitivity test but negative for oxidase, potato rot, and arginine hydrolase tests. However, only 15 of the 26 isolates were positive for levan production and one isolate recorded positive for potato rot test also. Therefore, on the basis of LOPAT test results, Lelliot and Stead (1987) grouped the pathovars of *P. syringae* under group Ia of fluorescent pseudomonads. On the other hand, Sands *et al*. (1970) categorized phytopathogenic pseudomonads into four major groups on the basis of nutritional and physiological characters and placed *P. lachrymans* in group-I, which were distinguishable from other fluorescent pseudomonads by low growth rates, ability to induce hypersensitivity on tobacco, absence of arginine dihydrolase and ability to use relatively limited ranges of carbon sources. Watanabe and Ohuchi (1983) studied biochemical characters of 110 Japanese isolates of *P. syringae pv. lachrymans* pathogenic to cucumber, and found that all of these were positive for production of fluorescent pigment, growth in Cohn's solution, beta-glucosidase activity, production of Levan, liquefaction of gelatin, utilization of tartarate and sucrose, and hypersensitivity to tobacco but were negative for growth at 37 ^{0}C , arginine dihydrolase, oxidase, tryrosinase, casein hydrolysis, cotton oil hydrolysis, nitrate reduction and potato soft rot activity.

Bradbury (1986) summarized the biochemical description of *P. syringae pv. lachrymans* as positive for levan production from sucrose, tobacco hypersensitivity, catalase and hydrolysis of arbutin and aesculin, slow for gelatin liquefaction and negative for pyocyanin and intra-cellular pigment production, arginine dihydrolase, oxidase, starch hydrolysis, potato rot and production of 2-ketogluconate within 3 days from gluconate. Scortichini *et al*. (1995) reported that the capability of aesculin utilization by *P. syringae pv. lachrymans* disappeared after serial transfers on King's B medium as well as after the passage through non-host plants and as such suggested that carbohydrate utilization ability of the bacterium would vary according to the different environments in which it had lived. Variation due to frequent subculturing of *P. syringae pv. lachrymans* in utilization of glycerol, ribose, D-xylose, rhaminose, inositol, and mannitol has also been reported (Scortichini and Rossi, 1995).

In a comperative study of phytopathogenic pseudomonads, Braun-Kiewnick and Sands (2001) reported that *P. syringae pv. lachrymans* was positive for pectate lyase, beta-glucosidase, polygalacturonase, aesculin hydrolysis, arbutin hydrolysis, gelatin liquefaction, levan production and ice nucleation, and could utilize D-mannitol, insitol, D-sorbitol, trigonalline, D-quinate, erythritol, L(+) tartarate, glutarate and DL-glycerate but not adonitol, D(-) tartarate, L-lactate, anthranilate and DL-homoserine for growth.

Host range of the pathogen

Though species identification in pseudomonads is mainly based on LOPAT characters, yet knowledge of host and the type of symptoms produced often enable an investigator to make a preliminary judgment to identify the causal agent since most of the plant pathogenic pseudomonads are host specific (Braun-Kiewnick and Sands, 2001). Pathogenicity and host range of *P. syringae pv. lachrymans* has therefore, been studied by many workers from time to time.

After the first record of angular leaf spot disease on cucumbers by Smith and Bryan (1915), its occurrence on various other cucurbitaceous crops was reported from different countries. Smith (1946) for the first time recorded occurrence of angular leaf spot disease on honey dew melons in nature and observed that the pathogen isolated from diseased melons infected cucumbers under conditions of artificial inoculation, whereas the cucumber isolate of the pathogen proved pathogenic to honeydew melons as well. Similarly, angular leaf spot of black zucchini squash in nature, was for the first time reported by Ark (1954a) from California, USA and the squash isolate of the pathogen was reported to be pathogenic to cucumber, watermelon, honeydew melon and muskmelon. Under natural conditions, a severe out break of angular leaf spot of cucumbers in Michigan, USA was reported to have affected pumpkin, squash, cantaloupe and watermelon also (Anderson and Thornberry, 1938). In his extensive description of *P. syringae* pv. *lachrymans,* Bradbury (1986) reported that natural hosts of the bacterium include *Cucumis sativus*, *C. melo, C. melo* var. *indorus, C. anguria, C. dipsaceus*, *Citrullus lanatus*, *Cucurbita maxima*, *C. pepo* var. *melopepo, C. pepo* var. *medullosa*, *C. pepo* var. *condensa, Bryonopsis laciniosa*, *Lagenaria leucantha* and *Luffa acutangula.*

During the host-range studies in field and green house, Van Gundy and walker (1957b) reported that all the commercial and ornamental cucurbit species tested were infected by the bacterium with most severe foliage and fruit symptoms on west Indian gherkin (*Cucumis anguria*) and cucumber *(C. sativus)*, respectively. Hopkins and Schenck (1972) reported that watermelon isolate of *P. syringae pv. lachrymans* proved pathogenic not only to members of Cucurbitaceae family such as cucumber, muskmelon squash and *Luffa* but was also weakly pathogenic to some members of Leguminosae family as well as to tomato and *Nasturtium*. However, it has been reported that ability of many bacterial plant pathogens to

cause an assortment of reactions on non host plants when dosages more than 10^7 cfu per ml are used, lead to the misinterpretation of host ranges, whereas there is a general recognition that high dosages may produce hypersensitive reaction when intromitted into a non host plant (Brain-Kiewnick and Sands 2001). Ohuchi *et al*. (1980) reported that Japanese isolates of *P. syringae* pv. *lachrymans* were pathogenic to 12 crops in Cucurbitaceae such as *Benincasa cerifera* , watermelon , melon, cucumber and bitter gourd but not to 30 other crops of different families. Similarly, El-sadek *et al*. (1992) reported that all the isolates of *P. syringae* pv. *lachrymans* tested by them, were pathogenic only on cucurbits such as , cucumber, squash, watermelon, pumpkin and melons.

Staub and Grumet (1993) reported occurrence of *P. syringae* pv. *lachrymans* on wild cucurbit specie *Cucumis sativus* var. *hardwickii*, which is used as potential source of variation in cucumber breeding. Similarly, studies on 49 accessions of wild cucurbit species revealed that 16 wild species were susceptible to *P. syringae* pv. *lachrymans* with some quantitative differences in extent of disease (Kudela and Labeda, 1997). Bhat (2009) reported that besides cucumber (*Cucumis sativus*), the pathogen infected muskmelon (*C. melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), squash (*C. maxima*), wild melon (*Cucumis melo* fsp. *agrestis* var. *agrestis*), bottle gourd (*Lagenaria siceraria*), bitter gourd (*Momordica charantia* Poir) and sponge gourd (*Luffa acutangula*) under conditions of artificial inoculation but under natural conditions the disease was recorded on all these cucurbits except bottle gourd, bitter gourd and sponge gourd.

Survival of the pathogen

It is well known fact that plant pathogenic bacteria do not have spores adapted to carry them over long unfavourable periods but survive as vegetative cells with seed or propagative parts, in diseased plant debris, in soil or in living crop or weed plants (Leben, 1981a). *P. syringae* pv. *lachrymans* has been found to survive on seed, diseased crop debris and in soil by different workers. Available literature on this aspect is reviewed in case of each mode of survival.

Infected seed as source of survival

Casner (1918), based on his laboratory and green house tests, concluded that *P. syringae* pv. *lachrymans* was introduced into disease free fields with the infected seed. Gilbert and Gardner (1918) verified the work of Casner and reported that the bacterium survived on the exterior of the seed, which appeared to be disease free after a period of twenty months in storage. Contrary to this, the bacterium has been reported to be confined not only to the seed surface but deep in seed tissues where it resists the seed treatment (Gorlenko and Voronkevich, 1946). Similarly, Wiles and Walker (1951) demonstrated presence of the bacterium in placental tissue and funiculus of the seed and also reported that the bacterium caused internal invasion of the seed at the time when infection progressed in infected cucumber fruits. The bacterium was isolated from 60 per cent seeds of the infected fruits, out of which 16 per cent of the seeds were reported to carry the pathogen internally (Kritzman and Zutra, 1983b).

Jones and Doolittle (1921) reported that the bacterium survived 20 months but not 32 months on the seed and thus two year old seed could not be considered pathogen free, while three year old seed apparently did not carry infection. Planting one year old seed obtained from previous years diseased fruits in fields not previously planted to cucumbers were reported to initiate epidemics of angular leaf spot disease at any time during the season, when temperature and moisture were favourable for the disease (Wiles and Walker, 1952).

Leben (1981b) reported that the seeds obtained from cucumber fruits artificially inoculated with *P. syringae* pv. *lachrymans* in field, yielded the pathogen after plating in selective media and the bacterium appeared to be located in the seed near the seed surface. The pathogen cells within the cucumber seed are in a state of low metabolic activity and in such a protected position that these were hard to be reached by usual treatment procedures. The healthy seeds could also be contaminated artificially by vacuum infiltrating the bacteria and such seeds produced 60 to 80 per cent diseased seedlings (Leben, 1983a). Bhat (2009) reported that two years' old seed is not free from the disease with the seedlings emerging out of such seeds bearing cotyledonary lesions and the pathogen can be retrieved from both naturally infected as well as artificially inoculated seed for more than 21 months after harvesting.

Survival on infected crop debris and soil

Longevity of *P. syringae* pv. *lachrymans* on diseased crop debris has been reported to depend on seasons when cucumber vines were buried in soil. For example, in summer, the bacterium died within 10 to 20 days, whereas its longevity was 3 to 4 months in winter, thereby indicated that soil transmission of the disease occurred in winter when the period of fallowing in cucumber fields was within 3 to 4 months (Watanabe and Ohuchi, 1983). Gilbert and Gardner (1918) reported occurrence of angular leaf spot disease in previous years diseased fields even when treated or disease free seeds were used and thus concluded that the bacterium over-wintered in infected soil upto the next sowing time. Gorlenko and Voronkevich (1946) reported that the bacterium over-wintered on diseased leaves left on surface of soil but not on the leaves buried by autumn tilling. The bacterium did not persist on diseased leaves left on soil surface beyond one or two years due to their decomposition after this period and the diseased fields were safe to be planted by cucumbers after a period of two to three years. Reports reveal that angular leaf spot pathogen remained viable in dry leaf tissue for two and a half years and also persisted in diseased plant debris in soil for varying periods ranging from 3 to 12 months (Van Gundy and Walker, 1957b; Kagiwata, 1991). Kritzmen and Zutra (1983a) reported that the pathogen was the poor survivor in the soil as its population declined to undetectable level after 8 weeks in inoculated soil, which was kept dry but persisted for over 90 weeks in the wetted soil containing diseased cucumber debris. Bhat (2009) reported that pathogen survives on crop debris beyond the next sowing time though its population on

debris buried under soil declined sharply as compared to that left on the surface.

Management of the disease

Management through 'Induced Systemic Resistance' (ISR)

Different workers have tested various physical, chemical and biological agents for their ability to induce resistance in crop plants against the attack of their potential pathogens. From the literature it is apparent that plants can be protected against fungal, bacterial and viral diseases by mechanisms, which resemble immunization in animals (Kuc and Hammerschmidt, 1978). Caruso and Kuc (1979) reported that infection of the first true leaf of a susceptible cucumber cultivar with either *P. syringae* pv. *lachrymans* or *Colletotrichum lagenarium* systemically protected it against the disease caused by subsequent challenge with either pathogens and the protection was evident even 37 days after the initial infection . Presence of necrotic tissue near sites of initial inoculation has been reported necessary for such kind of protection to occur (Caruso, 1977). Prior infection of first few leaves of cucumber by tobacco necrosis virus (TNV) or *P. syringae* pv. *lachrymans* has been reported to induce systemic resistance in plants against subsequent attack of *Cladosporium cucumerinum*, *P. syringae* pv. *lachrymans* and *Colletotrichum lagenarium* (Jenns *et al.,* 1979; Jenns and Kuc, 1980). Doss and Havesi (1981) reported that infection and induction of necrosis on first true leaves of cucumber by *Colletotrichum lagenarium* or treatment with mercuric chloride induced systemic resistance in upper leaves against angular leaf spot (*P. syringae* pv. *lachrymans)* used as challenge. They further reported that only the symptoms of disease were suppressed in upper leaves but the bacterial multiplication was not altered. Electrophoretic analysis of extracts of cucumber leaves infected with *Colletotrichum Lagenarium*, *P. syringae* pv. *lachrymans*, TNV, *Fusarium oxysporum* f.sp. *cucumerinum*, *Erwinia tracheiphila* or cucumber mosaic virus revealed the presence of a protein band on polyacryl amide gel, which was not evident in extracts of healthy or mechanically wounded leaves or in uninfected leaves of the infected plants but was detected in similar amounts in infected and secondarily challenged leaves of infected plants (Gessler and Kuc, 1982). These results point towards the fact that pathogenicity related (PR) proteins are synthesized in plants due to activation of defense genes in response to the pathogen attack.

Gottstein and Kuc (1989) reported that spraying undersides of first and second true leaves of cucumber with solutions of potassium oxalate, dibasic potassium phosphate, tribasic potassium phosphate, dibasic sodium phosphate, tribasic sodium phosphate or calcium hydrogen phosphate induced systemic resistance in leaves 3 and 4 of cucumber and newly developing leaves above leaves 3 and 4, which lasted for at least 5 weeks in green house and out door tests. The induced resistance was associated with appearance of chlorotic and necrotic spots on leaves 1 and 2, lack of which or rapid death of leaves 1 and 2 was associated with little or no induced systemic resistance. Besides these, spraying aqueous solutions of oxalic acid, or inoculating leaf 1 with a spore suspension of *C. lagenarium* has also been reported to induce systemic resistance to *P. syringae* pv. *lachrymans* and some other potential pathogens of cucumber when plants were challenged on leaf 2 with the test pathogens. Plants with distinct but restricted necrotic lesions on inducer leaves were better protected than those with extensive damage or few lesions (Mucharromah and Kuc, 1991).

Meuwly *et al*. (1994) reported that there was many fold increase in endogenous salicylic acid content of cucumber plants after they were inoculated on leaf 1 by *P. syringae* pv. *lachrymans* and this resulted in higher chitinase activity in leaves of such plants. An increase upto 21 fold in levels of salicylic acid in leaf 1 and 2 of cucumber was recorded 7 days after inoculation of *P. syringae* pv. *lachrymans* on lower surface of leaf 1 (Molders *et al.,* 1995). Various non-pathogenic microorganisms have also been reported to induce systemic resistance against many plant diseases. Seed bacterization or cotyledon infection with plant-growth promoting rhizobacteria (PGPR) such as, *Pseudomonas putida, Serratia marcescens, Flavomonas oryzihabitans* and *Bacillus pumilus* induced systemic resistance in cucumber against bacterial angular leaf spot (*P. syringae* pv. *lacrymans)* which resulted in significant decrease in lesion number and size, and in pathogen population in inoculated leaves (Liu *et al.,* 1995: Wei *et al*., 1996).

Raupach and Kloepper (1998) reported that level of systemic resistance induced by seed treatments with PGPR *Bacillus pumilus, B. subtilis* and *Curtobacterium flaccumfaciens* in cucumber against angular leaf spot and anthracnose under field conditions was higher when the PGPR were used in mixtures as compared to that when tested individually. In their further work, the authors reported that mixtures of PGPR were able to induce systemic resistance and offer significant protection with or without use of soil fumigant methyl bromide also (Raupach and Kloepper, 2000). Wang *et al.* (2000) reported that application of benzothiadiazole induced systemic resistance in muskmelon against angular leaf spot and powdery mildew, with two applications being more effective than one application.

Evaluation of chemicals *in vitro*

Morgan and Goodman (1955) reported that Neomycin inhibited growth of the *P. syringae* pv. *lachrymans in vitro* at a minimum inhibitory concentration of 0.1 µg ml^{-1}, streptomycin and Agrimycin-100 both at 0.2 µg $ml^{-1,}$ followed by terramycin (oxytetracycline), aureomycin, polymixin and streptothricin, respectively, whereas viomycin and chloromycetin inhibited the growth only at a higher minimum inhibitory concentration of 1.6 and 6.3 µg ml^{-1}, respectively. Streptomycin and tetracycline have been reported to significantly inhibit *in vitro* growth of *P. lachrymans* in plate tests by Knosel (1965). On the other hand, Mukai *et al.* (1976) reported that out of 121 isolates of *P. syringae pv. lachrymans* collected from all over Japan, 52 isolates showed resistance to even very high concentrations of streptomycin and chloramphenicol. However, the proportion of isolates which exhibited resistance to streptomycin and dihydrostreptomycin had increased to 77.3 per cent in Japan during 1978 (Yano *et al.,* 1978). Similarly, Kagiwata (1992) reported that many

Japanese isolates of *P. syringae pv. lachrymans* showed varying levels of resistance to streptomycin, chloromycetin, kanamycin and terramycin (oxytetracycline). Therefore, in a search to find some effective alternative to streptomycin in Japan, Sakurai *et al.* (1977) reported that the streptomycin-resistant isolates were sensitive to Kasugamycin with the exception of two isolates. Myomycin was also found to be an effective antibiotic which inhibited *in vitro* growth of both streptomycin-sensitive and streptomycin-resistant isolates of *P. syringae pv. lachrymans* in Japan (Yoneyama *et al*., 1978).

Khlaif and Abu-Blan (1994) reported that in laboratory tests, Agrimycin-100, copper oxychloride, streptomycin sulphate and trimiltox (mancozeb +copper oxychloride+ copper carbonate) inhibited growth of *P. syringae pv. lachrymans* by 96.7, 84.3, 76.7 and 87.7 per cent, respectively. Protease activity of *P. syringae pv. lachrymans*, which has a role in its pathogenicity, was also greatly reduced by the addition of thiabendazole, cumazin, terramycin or streptomycin to the growth media (El-Sadek *et al*., 1992). Bronopol has also been reported to exhibit a very high degree of antibacterial activity against *P. syringae pv. lachrymans* and some other bacterial plant pathogens *in vitro* (Klimach *et al*., 2001).

Seed treatment

Casner (1918) reported that angular leaf spot of cucumber was introduced into fields with the infected seed and suggested seed disinfection as an effective control measure to prevent the disease. Gilbert and Gardner (1918) reported that immersion of cucumber seeds infected with angular leaf spot pathogen in 1: 1000 mercuric chloride solution for five minutes followed by fifteen minutes washing proved most satisfactory from the stand point of safety and effectiveness in preventing the disease and therefore, recommended this treatment for commercial use. Reports reveal that use of Aresan and mercuric chloride at different concentrations and hot water at 50 ^{0}C for 30 minutes as seed treatments proved about equally effective, but each treatment reduced the percentage of infected seedlings to only about one-half compared to those from untreated seed and therefore, had a negligible effect on epidemic development when favourable temperature and moisture prevailed during the crop growth (Wiles and Walker, 1952; Middleton and Bohn, 1953). Waksman (1949) reported that cucumber seed contaminated with *P. lachrymans,* when soaked in streptomycin solution at 1: 10000 for 20 minutes and dried before sowing resulted in cucumber plants with no infection as against 5 per cent infected plants in case of untreated check. Similarly, Severin *et al*. (1971) reported that hot water treatment above 54 ^{0}C eliminated cotyledon infection but longer exposure times delayed seed germination, whereas immersion in streptomycin at 80 to 100 ppm for 15 minutes gave good disease control but oxytetracycline proved less effective. Stanek (1958) also reported that soaking cucumber seeds in 0.05 to 0.4 per cent Fytostrept (streptomycin + terramycin) for 1.5 to 6 hours was successful against *P. syringae pv. lachrymans* and stimulated seedling growth. Marras and Corda (1973) reported that infection of *P. lachrymans* from squash seed was eliminated by hot water treatment at 50 ^{0}C for 30 minutes, immersion in mercuric chloride at 0.1 per cent for 10 to 20 minutes or in 1 per cent acetic acid for 1 to 2 hours.

Kutova and Filipova (1976) reported that soaking of cucumber seeds for 18 hours in zinc sulphate or manganese sulphate at 0.02 per cent, copper sulphate or boric acid at 0.03 per cent or in pencillin or streptomycin at 0.04 per cent greatly reduced infection of *P. syringae pv. lacrymans* and markedly increased field germination, root length and seedling viability with 4 to 16.4 per cent increase in fruit yield. Similarly, Kutova and Vlakhov (1977) reported that wet treatment of cucumber seeds with antibiotics tetracycline, oxytetracycline, C-7/21 and C-06 reduced angular leaf spot infection and increased field germination by about 10 per cent.

Umekawa and Watanabe (1978) reported that disinfection of cucumber seeds by dry heat at 70 ^{0}C for 3 days or by hot water treatment at 52 ^{0}C for 10 minutes or 54 ^{0}C for 5 minutes gave excellent control of *P. syringae pv. lacrymans* and only slightly reduced percentage germination, whereas chemical seed disinfection was less effective. Contrary to this, Leben and Sleesman (1981) reported that hot water treatment at 52 ^{0}C for 10 minutes and dry storage at 70 ^{0}C for three days reduced seedling infection only by 34 and 75 per cent, respectively, whereas seedling infection was prevented or greatly reduced when artificially infected seeds were stored at 50 ^{0}C and a relative humidity of 75 per cent for 3 days. Marinescu (1982a) reported that treatment of cucumber seeds by vacuum infiltration with Cryptonol (potassium hydroxyquinoline sulphate) at 0.1 per cent or kocide 101 (copper hydroxide) at 0.5 per cent gave a good control against the primary infection of *P. syringae pv. lacrymans*. In his further work Marinescu (1982b) reported that vacuum infiltration of cucumber seeds with copper hydroxide at 0.25 per cent, cuprous oxide at 0.5 per cent, oxytetracycline at 0.05 per cent, chinosol at 0.1 per cent or streptomycin at 0.05 per cent significantly controlled *P. syringae pv. lacrymans* on the seeds.

In Japan, streptomycin solution was not found useful due to wide distribution of streptomycin-resistant strains of *P. syringae pv. lacrymans* , and as such soak treatments of infected seeds in sodium hypochlorite solution at 1: 20 for 20 minutes or calcium hypochlorite at 0.25 to 0.5 per cent for 60 minutes were recommended as effective seed treatments against the disease (Watanabe and Ohuchi, 1983).

Chemical control in field

Many workers have reported the efficacy of many copper compounds to control the spread of angular leaf spot of cucumber under field conditions. Hellmers (1950) reported that cucumber plants inoculated with *P. lachrymans* remained healthy after spraying with 1 percent Bordeaux plus 0.5 per cent resin soap, while unsprayed pants were completely destroyed in 14 days. Similarly, Beecher and Doolittle (1950) reported that sprays with tribasic copper sulphate gave almost a complete control of disease spread in a field but Ziram and Zineb failed to control the disease spread.

Naumann (1965) reported that 5 to 7 sprays at weekly intervals or 3 to 4 sprays fortnightly with Spritzcuprol-45 (copper oxychloride) at 0.5, 0.75 and 1 per cent proved most effective in control of angular leaf spot of cucumbers under field conditions. The treatment at 1 per cent before infection proved better than a later application. In his further work, Naumann (1968) reported that the efficacy of Spritzcuprol-45 to control angular leaf spot of cucumber improved by using the sticker Fakema at 0.2 per cent or mineral oil at 1 per cent along with it. Similarly, Miller (1973) reported that 4 sprays at weekly intervals starting at 5 to 6 leaf stages with Agrimycin, copper hydroxide or copper hydroxide plus mancozeb reduced the number of angular leaf spot lesions on leaves but none gave economic control of the disease. Control of angular leaf spot by copper oxychloride was reported to improve by shortening spray intervals to 4 or 5 days so that all new emerged leaves received spray during the critical 4 to 6 days when they were more susceptible, and the increased costs through higher spray frequency were compensated by using low volume mist blowers instead of high volume knap-sac sprayers (Mabbett and Phelps, 1984).

On the basis of glass house tests, the copper fungicides were reported unable to provide adequate protection against the angular leaf spot disease but the cucumber plants were reported to remain almost disease free by three sprays of streptomycin and tetracycline at 200 ppm with streptomycin stimulating the growth, while oxytetracycline causing slight growth depression which was compensated by its excellent bactericidal effect (Knosel, 1965). Similarly, Ark and Wilson (1956) reported that in pot experiments, nuclay and hydrated lime-streptomycin formulations gave good control of angular leaf spot of cucumber when dusted on both sides of the leaf prior to mechanical inoculation. Foliar sprays with Agrimycin-100 (streptomycin + terramycin) have also been reported to give good control of angular leaf spot of cucumber under field conditions and proved superior over terramycin when used individually (Doolittle and Beecher, 1955).

Watanabe and Ohuchi (1983) reported that spray of Bordeaux mixture and inorganic copper compounds held great importance against the streptomycin resistant strains of the pathogen as these proved useful to protect the vines and fruit of cucumber from angular leaf spot disease but inflicted slight phytotoxic lesions on the sprayed vines. Gornick and Amselem (1992) reported that sprays of Funguran (copper oxychloride) were effective in controlling angular leaf spot of cucumbers. On the other hand, Khlaif and Abu-Blan (1994) reported that sprays with Trimiltox (a mixture of mancozeb, copper oxychloride and copper carbonate) and cuprosan (copper oxychloride) were effective against angular leaf spot of cucumber when used prior to infection but did not give curative results. In his further work Khlaif (1995) reported that Ridomil and Trimiltox Forte (copper oxychloride plus mancozeb) were most effective in decreasing incidence and severity of angular leaf spot with an increase in cucumber yield. Klimach *et al*. (2001) reported that under field and green house conditions application of bronopol at 0.01 and 0.02 per cent significantly decreased the intensity of angular leaf spot of cucumbers induced by *P. syringae* pv. *lachrymans.*

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