

# Pathological and physiological studies on spot blotch of barley caused by *Helminthosporium sativum* in A.R.E

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Barley is one of the important cereal crops in the world. Helminthosporium diseases are considered as the most destructive diseases which attack this crop. In this respect spot blotch became one of the main causes of yield reduction of barley in Egypt, especially in warm, wet weather in coastal regions. The causal organism was isolated from different localities in Egypt, identified and the pathogenicity was determined. The host range and viability of the pathogen were studied together with the activities of pectolytic, cellulolytic and oxidative enzymes for both the pathogen and extracts of barley plants. The relationship between resistance and chemical components of barley plants i.e. amino acids, sugars and phenolic compounds and the toxic effect of culture filtrates of the fungus were evaluated. The susceptibility and resistance of different barley varieties to the disease was studied. The effect of different fungicides on linear growth of the pathogen in laboratory and in the greenhouse and the field was studied also. Results obtained could be summarized as follows: (1) Spot blotch of barley in Egypt during seasons 1979-81 indicated that the disease was found in 154 coastal regions, Delta and the Middle Governorates of Upper Egypt. The highest percentages of infection were observed at Kefr El-Sheikh, Alexandria, Damietta and decreased gradually southwards. (2) Twelve isolates of *Helminthosporium sativum* were isolated from diseased leaves showing typical spot blotch symptoms. The pathogenicity test revealed that the selected isolates varied greatly in their pathogenicity together with differences in morphological characteristics and isolate No. 4 was the most virulent isolate, whereas isolate No. 12 was the least virulent one. (3) Results of the host range of *Helminthosporium sativum* indicated that the fungus has a wide host range in Egypt, as it was found to infect *Avena sativa*, *Cynodon dactylon*, *Lotus*, *Echinochloa colonum*, *Chenopodium*, *Hordeum vulgare*, *Lolium temulentum*, *Linum catharticum*, *Oryza sativa*, *Polypogon monspeliensis*, *Setaria viridis*, *Sorghum vulgare*, *Triticum vulgare* and maize. (4) The severity of the fungus increased gradually and reached the maximum after 1 month, then gradually decreased by ageing till the 18th month. (5) The highest activities of polygalacturonase (PG), Cellulolytic (ex) and pectinmethylesterase (PME) enzymes were found in the fungal filtrates of isolate No. 4 (the most virulent) whereas the lowest activities were in isolate No. 12 filtrate (the less virulent). (6) As regards the effect of fungicides on enzyme activities studies indicated that PG and ex activities were reduced with the increase in the concentration of the tested fungicides specially in case of plantavax and bayleton, whereas Wolfen-thiram and Dithane M22 had little effect on PG and ex enzymes activities. (7) Polyphenoloxidase, Peroxidase, Catalase and Ascorbic acid oxidase activities were relatively higher in the mycelial matrix than in the filtrate of each of the tested isolates. Also these activities were higher in the highly pathogenic isolates (isolate No. 4) than the least pathogenic one (isolate No. 12). These activities increased also by increasing the incubation periods in both mycelial matrix and fungal filtrate. (8) Adult plants showed high levels of polyphenoloxidase activity as compared with the seedlings. In this respect infection with *Helminthosporium sativum* caused gradual increase in enzyme activity specially in the resistant varieties especially in the seedling stage. (9) Adult plants and seedlings of resistant varieties exhibited higher levels of peroxidase activity as compared with the susceptible ones. In this regard the increase of peroxidase

idase activity was higher in susceptible varieties and higher resistant ones specially in adult plants. (10) Catalase activity was higher in seedling and adult leaves of the tested varieties specially in resistant ones. Also, infection with the pathogen exhibited higher catalase activity in the resistant cultivars than in susceptible ones. (11) Healthy leaves from seedlings and adult plants of the resistant varieties exhibited higher activity of ascorbic acid oxidase enzyme than that in the susceptible ones. Inoculation with *H. sativum* resulted in an increase in ascorbic acid oxidase enzyme activity specially in the leaves of resistant varieties than in that of susceptible ones and in adult plants than in seedling stage. (12) Concerning phenolic compounds, results indicated that total, free, conjugated and ortho-dihydroxyphenols were found in higher amounts in the resistant varieties than in susceptible ones. However, it was found clearly that phenolic compounds accumulated faster in resistant varieties than in susceptible ones as a result of infection and also by long incubation periods specially after 10 days from inoculation and in resistant plants. (13) Total and reducing sugars contents were higher in leaves of susceptible varieties than in the resistant ones. Adult plants contained higher amounts of total and reducing sugars than the seedlings. Inoculation with *H. sativum* caused pronounced decrease in both total and reducing sugars in seedlings or adult plants of susceptible varieties, whereas, inoculation induced an increase in the resistant ones. (14) Paper chromatographic analysis showed the presence of Maltose, Sucrose, Glucose, Galactose and Fructose in inoculated and uninoculated leaves of all the tested varieties in both seedlings and adult plants. However, no clear correlation was found between resistance or susceptibility of the tested barley varieties and any of the above-mentioned sugars. (15) Concerning total free amino acids, their content increased as a result of inoculation with *H. sativum* in both resistant and susceptible varieties in the seedlings as well as in adult plants. In this regard, susceptible varieties contained higher levels of total free amino acids than the resistant ones in both seedling and adult plant stages. However, adult plants contained higher amounts of amino acids than seedlings. (16) Qualitative analysis indicated the presence of seventeen amino acids namely Cystine, Lysine, Histidine, Arginine, Aspartic acid, Glycine, Serine, Glutamic, Threonine, Alanine, Proline, Tyrosine, Methionine, Valine, phenylalanine, Leucine and Iso-leucine in seedlings as well as adult plants of resistant and susceptible varieties. (17) The amounts of total amino acids increased as a result of infection in both the susceptible and resistant varieties as compared with the uninoculated control. (18) The quantities of certain amino acids as Lysine, Histidine and Arginine were higher in the susceptible varieties than resistant ones, whereas the contrast was noticed as regards Tyrosine and phenylalanine. On the other hand, Proline appeared with higher concentrations in the susceptible varieties than the resistant ones in both seedlings and adult stages and its concentrations were higher in healthy plants as compared with inoculated ones. (19) In response to inoculation with *H. sativum*, Lysine and Phenylalanine quantities increased in the resistant varieties and decreased in the susceptible ones. In this respect, Methionine and Valine increased as a result of infection in resistant varieties in the seedlings. The highest amounts of Lysine, Histidine and Arginine were obtained in the infected susceptible varieties. As for, the other amino acids no general trend was noticed in either resistant or susceptible varieties. (20) The culture filtrate of two isolates of *H. sativum* (one virulent and the other avirulent) induced inhibitory effect on the percentage of seed germination, lengths of coleoptile and roots on all the tested varieties. However, the high concentration of crude culture filtrate was the most effective in this respect. This effect decreased proportionally with the dilution of crude filtrate. In this regard the most effective filtrate was taken from 30 days old cultures and was that of the most virulent isolate No. 4. (21) One of the fractions was isolated in crystalline form (rod crystals), according to the method adopted by Lindberg (1971), and Turner (1971) and was identified as Helminthosporin. In this regard the virulent isolate No. 4 produced 4.633 g/liter of culture medium of toxin; while the less virulent isolate No. 12 produced 2.461 g/liter of culture medium. The toxin gave similar symptoms on seedlings and adult plants and effects as mentioned before. However, the phytotoxic effect of the toxin decreased proportionally by decreasing its concentration. (22) Screening of 37 selected barley varieties under greenhouse conditions in the seedlings, indicated that 16 varieties were highly resistant and 5 varieties were moderately susceptible, while 16 varieties were susceptible. On the other hand, under field conditions in adult stage results indicated that from 44 varieties, 19 were

resistant, 4 moderately susceptible, 13 susceptible and 8 were very susceptible. (23) As regards the correlation between the behavior of 45 varieties in both seedling and adult stages, it was found that these varieties could be put into two groups as follows: A- The first group in which there was no effect of plant age on the varietal resistance or susceptibility as follows: 1. Seven varieties were resistant in both seedlings and adult stages. 2. Fourteen varieties were susceptible in both seedlings and adult plants. B- The second group in which there was an important effect of plant age on the varietal resistance or susceptibility as follows: 1. Eleven varieties showed increasing resistance with age. 2. Six varieties were susceptible in the seedling stage and became resistant with age. 3. Seven varieties showed increasing susceptibility with age. (24) Screening fungicides *in vitro* showed that the tested fungicides varied in their effect on fungal growth. However, all the fungicides except Bavistin and Spergon almost stopped the linear growth of *Helminthosporium sativum* at concentrations of 100 ppm. This effect increased with the increase in concentrations. The fungicides Wolfen thiram, Dexon, Plantavax and Dithane M completely inhibited the fungal growth at the concentrations of 50, 200, 400 and 400 ppm respectively. (25) Using these fungicides as seed dressings under greenhouse conditions increased the percentage of seed germination, the number of ears per pot, the weight of grains in gm per pot and 100 grains weight. On the other hand, the severity of infection was reduced as the fungicides were applied. However, Wolfen thiram, Dexon and Spergon were superior in this respect while Vitavax and Brassteol followed the aforementioned fungicides in their effect, whereas Bavistin was almost ineffective. (26) Using these fungicides as foliage spray under greenhouse conditions (after artificial infection by spraying with a spore suspension at the concentration of 80,000 - 100,000 conidia/ml. of *Helminthosporium sativum*), the severity of infection was reduced while the weight of grains per pot and 100 grains weight significantly increased. Plantavax and Dithane M45 (Mancozeb) were superior in this respect while Dithane M22 (Mancozeb) and Bayleton followed them. (27) Using these fungicides as seed dressings under field conditions showed that all the tested fungicides reduced the severity of infection and significantly increased the yield and weight of 1000 grains. In this respect, Wolfen thiram, Dexon and Vitavax were superior to the other tested fungicides. On the other hand, by using fungicides for foliage spray under field conditions after artificial infection by spraying spore suspension of *Helminthosporium sativum*, the severity of infection was reduced as the fungicides were applied where a significant increase in the yield for each plot and the weight of 1000 grains was noticed. Dithane M45 and Plantavax were superior in this respect to the other tested fungicides.