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EFFECT OF CLIMATIC CONDITIONS ON LIFE CYCLE OF CHARCOAL ROT INFECTED SUNFLOWER PLANT

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Abstract

Sunflower (*Helianthus annuus* L.) belongs to the family *Asteraceae*. *Helianthus. annuus* ssp. *lenticularis* (wild), *H. annuus* ssp *annuus* (weed) and *H. annuus* ssp *macrocarpus* cultivated. *H. annuus* ssp *macrocarpus*, the giant sunflower, is cultivated for edible seed. Climatic conditions like temperature, atmospheric humidity and available moisture play a significant role in activation and multiplication of *Macrophomina phaseolina*. High moisture and low temperature favour the rapid spread of the fungus at seedling stage. Sunflower seed was surface sterilized with 5% chlorox. In greenhouse studies, NPK fertilizers were not applied. There was no significant difference in germination period of plants grown under field and greenhouse conditions. The plants infected with *Macrophomina phaseolina* grown under greenhouse conditions completed all stages of its life cycle appeared after seedling stage earlier than plants cultivated under field conditions during spring and autumn seasons. *M. phaseolina*-infected plants approached seedling stage five days earlier than the plants grown under field conditions. Vegetative, reproductive, maturity stage 10 days after maturity appeared 15, 5, and 7 days earlier than charcoal rot infected spring crop grown under field conditions. Inadequate food translocation affects physiological processes like length of growth stages of the plant. Plants raised in greenhouse were affected by charcoal rot at earlier stages of plant growth.

Introduction

Low yield of sunflower in Pakistan can be attributed to several biotic and abiotic constraints. The important factors are lack of production of good quality seed for cultivation and lack of postharvest treatment facilities. Pests and diseases play a major role in this regard. Rana (1999) and Zimmer & Hoes (1978) reported that on average diseases cause loss of 12% in yield to sunflower from nearly 12 million hectare in the world. Until now more than 25 diseases have been reported on sunflower in China (Xiang *et al*., 1978). *Alternaria helianthi, Orobanche coerulescens, Sclerotinia sclerotioum and Septoria helianthi* cause 10-15% yield losses. Madjidiech, (1988) reported 20-50% losses due to *Plasmopara helianthi* on sunflower in Iran. Mirza & Beg, (1983) conducted first survey of the sunflower crop in the central and northern areas of Pakistan during 1982. In this survey, *M. phaseolina* (charcoal rot) and *Rhizopus spp.* (head rot) were reported as most destructive on sunflower crop. Mirza, (1984) reported yield losses due to *M. phaseolina* up to 90% in Pakistan on sunflower.

Charcoal rot of sunflower was reported for the first time from Faislalabad (Mirza, 1984) and later from other areas of Punjab, Sindh and NWFP provinces as a serious threat to sunflower (Mirza & Beg, 1983; Steven *et al.*, 1987). In Pakistan charcoal rot is associated at maturity of plants under water stress. Favorable conditions for the development of the pathogen may lead to the death of plant or failure of the crop. Up to

60% yield losses due to charcoal rot have been reported (Steven, *et al*., 1987). The fungus is reported to be soil, seed and stubble borne. The evidence suggests that it is primarily a root inhibating fungus and produces tuber or cushion shaped 1-8 mm diameter black sclerotia. These sclerotia serve as a primary means of survival (Smith, 1969; Mirza, 1984; Kaisar *et al*., 1988). *M. phaseolina* has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species (Indera *et al.,* 1986). So far in Pakistan, 67 economic hosts of *M. phaseolina* including cotton, rice, maize, cucurbits, okra, and wheat have been reported (Mirza & Qureshi, 1982; Shehzad *et al*., 1988). Wide host range of *M. phaseolina* suggests it as non-host specific fungus. Physiological specialization of the fungus is not well demonstrated. High level of variation in morphology, physiology and pathogenesis has been reported even when isolated from different parts of the same plant (Dhingra & Sinclair, 1973). High level of variation in fungus, soil borne habitat, and good survival ability of the sclerotia makes its chemical control difficult and uneconomical. Therefore, the most appropriate approach to combat the pathogen is the use of resistant varieties. So far no commercial sunflower cultivar has been reported for resistance against *M. phaseolina* (Gul *et al*., 1989; Ahmed & Burney, 1990; Hafeez & Ahmad, 1997). The objectives of the present studies were to identify role of climatic factors in disease management

Materials and Methods

Nursery raising: Keeping a 5 cm free board a seedbed of 7 cm thickness was prepared with sterilized river sand in 30 x 45 cm plastic trays. Sand was sterilized at 120^{o}C at lb/inch^{2} for 60 minutes. Before sterilization sand was passed through 20-mesh sieve to avoid interference of foreign elements in germination. Few holes were made at bottom of the tray for drainage of excessive moisture. Water was sprinkled to make approximately 75% moisture level of seedbed 5-6 hours before planting the seed. Seed were sown at 1-2 cm depth keeping 10 plants in a row, 5-cm row to row and with 2.5-cm plant-to-plant distance. A block of three rows was allocated for each of the test isolates. A partition wall was made for separating the blocks of isolates with the help of a polyethylene sheet. The seed trays were kept at room temperature with a range of 20-28 $^{\circ}$C and 12 hours daylight for 7-10 days. On achieving the required length, central row of the block was inoculated with a sclerotial suspension. Due to variation in size of scleroia among the test isolates, the sclerotial suspension was made by agitating approximately 150-200 sclerotia in 100 ml of distilled sterilized water. A hole of 5-7 mm diameter was made close to the root zone of the plant with the help of a plastic rod. The sclerotial suspension was applied gently in the hole and closed after application of sclerotia. Seed trays were kept under climatic conditions favorable for development of *M. phaseolina*; i.e., high temperature (30-32°C) with slight moisture stress (40-45%). Forty-eight hours after inoculation, plants were randomly examined at 24-hour intervals for development of infection. A 7-10 days after inoculation, fresh seedling tissue was plated on a 90 mm Petri dish containing PDA and incubated in dark at 30 ± 2°C for 3-5 days. Before plating, seed specimens were processed as discussed under 3.3.

Selection of test material: Promising hybrids of sunflower were tested by NODP, NARC, Islamabad from 134 local and exotic origins during 1992-1994. Among these tested germplasm 18 hybrids (Appendix 8b) were selected as candidate hybrids for National Uniform Yield Trails (NUYT) because they had desired agronomic characters. These

candidate hybrids were evaluated for charcoal rot resistance against three selected isolates MP8, MP61 and MP70a of *M. phaseolina* because these isolates exhibited stable behavior in their aggressiveness during autumn 1997 and spring 1998 cropping seasons.

Greenhouse studies: The studies on the role of climatic conditions on the life cycle of charcoal rot infected plant, variation in host pathogen interaction, inhibitory effect of whole extracts of sunflower plant parts against sclerotial production of Macrophomina phaseolina, role of pathogen inoculum density on grain yield and plant mortality were conducted under greenhouse conditions. In these studies same criteria were used for selection of host and pathogen and methodology for raising plants. The variation in methodology for these experiments was either in sclerotial dose, time of application, and parameters for data recording.

Selection of host and pathogen: These studies were based on the previous findings for aggressiveness of pathogen and resistance of host. Three isolates MP8, MP61 and MP 70a, belonging to low and high aggressive subgroups were selected for detailed studies on pathogenic behavior of *M. phaseolina*. These isolates exhibited stable aggressive reaction in field studies during autumn 1996 and spring 1997. Similarly stability in the resistance reaction was observed in sunflower hybrids. The hybrids LG Slobel and Tarnab 713 were highly resistant and Parsun 1 was highly susceptible against MP8, MP61, and MP 70a isolates of *M. phaseolina* during spring 1997 and autumn 1998 under field conditions.

Laboratory scale inoculum production: Three selected isolates MP8, MP61 and MP 70a were cultured on Soybean dextrose broth (SDB) (Appendix-5). In a 400ml autoclavable glass jar, 120 ml SDB was placed. The jars were autoclaved at 120°C, lb/inch2 for 30 minutes and cooled at room temperature (40-45°C). A 5 mm disc from the periphery of actively growing test culture was placed on longitudinally laid SDB jars and incubated in dark at 30 ± 2°C for 15-20 days, until a thick sclerotial mat developed on the broth surface. During the incubation period the jar cap was slightly loosened after 3 – 4 days to create aerobic conditions. The sclerotia mat was air dried at room temperature for 24 to 48 hours on a blotter sheet. Dry sclerotia mat was grinned in a find polished clay pestle mortar. Five grams of ground sclerotia were mixed in 500 ml sterilized distilled water and blended in a Warling commercial blender (CB 6) for 3-5 minutes for homogenization and separation of medium proteins. Blended suspension was passed through sieve no. 60 and 170 under gently running sterilized distilled water and air dried for 24-48 hours at room temperature. Sclerotia was stored in Pyrex 13 x 100 ml capped glass tubes and placed at cool place for running experiments and stored at 4°C for long term storage (Dillard *et al*., 1995).

Filling of pots and seed planting: Six days before planting the seed of selective hybrids LG–Sloble, Tarnab–713 and Parsun 1, pots were filled 2/3rd with sterilized corn flour amended soil medium. The soil layer was covered with required concentration of inoculum (gm/kg soil) of the isolates MP8, MP61, and MP 70a. Before planting the seed, moisture capacity of soil was adjusted to an appropriate level. So that adequate aeration for germination and root growth should be available. Seed was planted at 1-2 cm depths and inoculum was covered with 1 cm layer of sterilized soil.

Results

The studies were conducted to compare the differences in appearance of germination, seedling, vegetative growth, reproductive (flower initiation and completion) and maturity stages appearing in sunflower hybrids under natural field conditions. The field experiments were conducted during spring 1997 and autumn 1998 in the experimental fields of NODP, NARC, and the crop was provided with routine agronomic inputs.

In greenhouse studies plants were grown in 25x38 cm earthen pots containing sterilized soil medium of fine river sand, garden peat and clay at 1:1:1 ratio. Sunflower seed was surface sterilized with 5% chlorox. In greenhouse studies, NPK fertilizers were not applied. Seven days before planting the seed, sclerotia of MP8, MP61, and MP70a isolates of *Macrophomina phaseolina* were applied @ 0.5g/Kg soil and sclerotia were covered with 3-5 cm thick layer of soil spread on the top layer of the soil. The plants grown in greenhouse were provided environments favorable for the growth and development of the fungus and data were recorded at various growth stages of plant (Fig. 1).

Effect of climatic conditions on life cycle of charcoal rot infected plant: There was no significant difference in germination period of plants grown under field and greenhouse conditions. During autumn and spring seasons in the field and under greenhouse conditions, germination of the seed took place in 18 days after planting. The plants infected with *Macrophomina phaseolina* grown under greenhouse conditions completed all stages of its life cycle appeared after seedling stage earlier than plants cultivated under field conditions during spring and autumn seasons. The *M. phaseolina*-infected plants approached seedling stage five days earlier than the plants grown under field conditions. Vegetative, reproductive, maturity and stage 10 days after maturity appeared 15, 5, and 7 days earlier than charcoal rot infected spring crop grown under field conditions.

Discussions

Like genetics of host and pathogen, environmental factors equally determine severity of infection in plants. The environmental factors stimulate or inhibit physical and biological process of host and pathogen for disease development. Soil factors like moisture index, soil chemistry, soil texture and structure, and penetration of solar radiation are more important than atmospheric environmental factors for soilborne diseases. Soilborne diseases negatively affect the translocation of food in the plant body because they affect the root system of the plant. Inadequate food translocation affects physiological processes of plant like length of growth stages of the plant (Agrios, 1978; Mavi, 1986; Ahmad, 1996).

Little information is available on the effect of soilborne disease on host physiology under variable environments. The studies were conducted to evaluate the role of environmental variation on growth physiology of charcoal rot-infected sunflower. Physiological changes were measured on length of germination, seedling, vegetative growth, reproductive phase (flower initiation, flower completion), and maturity phase in a set of environments. In the present studies, the importance of knowing the host-pathogen interaction on the growth physiology of plant was realized, irrespective of the genetic variation existing in host and pathogen population. Such studies may prove helpful in the disciplines of crop meteorological, pathological, and physiological for studies on biotic stress in sunflower.

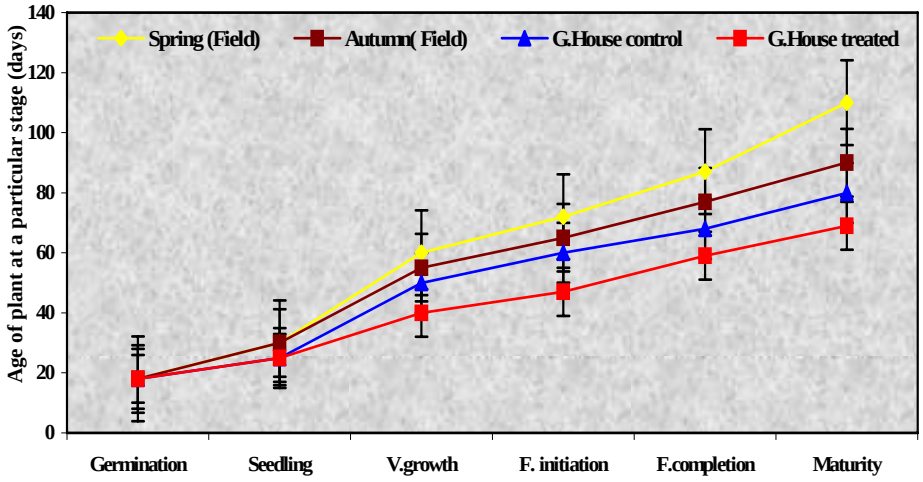

Fig. 1. Effect of climatic conditions on life cycle of charcoal rot infected sunflower

Findings of the studies are based on comparison of length of growth stages of sunflower under natural field conditions during spring and autumn season for two years and in the protected environment of greenhouse.

Due to the genetic potential of the hybrid, no significant difference was observed at germination and three-leaf stage of the plant raised under field or greenhouse environment. In greenhouse studies, sclerotia inoculum was mixed in the soil. As root came in contact with the germinating sclerotia, the fungus established itself in the root system and started to impair in the normal functioning of the plant. Plants raised in greenhouse were affected by charcoal rot at earlier stages of plant growth. Under natural soil conditions many microorganisms do react with fungus or its toxins, which effect aggressiveness of the fungus. But in sterilized soil under greenhouse conditions, there is no chance of reaction with competitive microorganism. Therefore, plants raised in the greenhouse were more severely exposed to charcoal rot because *M. phaseolina* established itself in the plant at earlier stages. Loss in resistance under greenhouse conditions was also supported due to absence of NPK fertilizers. Hindrance in normal functioning of the plant at early stages of plant growth resulted in early maturity of the crop. In comparison with the spring crop, plants raised in a greenhouse typically experience a significantly shorter life span from seedling to crop maturity.

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