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EFFECT OF DIFFERENT INOCULATION METHODS AND INOCULUM LEVELS OF *MACROPHOMINA PHASEOLINA* ON OKRA

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A B S T R A C T

Among two methods of *Macrophomina phaseolina* inoculation used for pathogenicity test, soil infestation method comparatively checked more plant growth of okra plants than seed infestation method. Minimum plant length and weight, as well as seed germination were observed by soil infestation method. Significantly maximum plant mortality and root infection was also occurred in soil infestation method. Seed germination, plant growth, plant mortality and root infection of okra plants were adversely affected with the increasing inoculum levels of *M. phaseolina*. Seed germination and plant growth were negatively correlated with inoculated pathogen population; whereas, plant mortality and root infection were positively correlated with the inoculum level of *M. phaseolina*.

Keywords: *Macrophomina phaseolina*, okra, inoculation methods, pathogenicity, inoculum density.

INTRODUCTION

Among different plant pathogens attacks okra (*Abelmoschus esculentus* L.) *Macrophomina phaseolina* (Tassi) Goid. is considered as one of the most destructive soil borne pathogen of okra (Hafiz, 1986). It has very wide host range causing diseases on more than 500 cultivated and wild plant species worldwide (Jones and Canada, 1994). In Pakistan, it causes infection on more than 67 economic hosts including field crops, pulses, flowers and vegetables (Khan, 2007; Mirza and Qureshi, 1978; Shehzad *et al.,* 1988). The fungus can also cause hallow stem, root rot, pre-emergence and post-emergence damping-off (Reuveni *et al.,* 1983). *M. phaseolina* is most often seen during summer weather (Gulya *et al.,* 2002). About 5-100% yield losses due to this disease have been reported (Vyas, 1981). *M. phaseolina* does not survive more than seven days in its mycelial form but it sclerotia can survive over ten months in soil (Ghaffar & Akhtar, 1968). It usually develops when soil temperature is 80-95^{o}F (27-35^{o}C) for 2 to 3 weeks (Yang and Navi, 2003). In most case, soil bone root infecting pathogens, the rate of disease development and disease severity is directly relating with the pathogen propagules present in the soils, as well as the mode of pathogen invasion (Madden, 1980; Kenerley & Bruck, I987). Keeping in view the yield losses caused by *M. phaseolina* in okra, the present investigation was initiated to evaluate the impact of different inoculation method and inoculum density of *M. phaseolina* on okra crop.

MATERIALS AND METHODS

M. phaseolina was isolated from roots and stem of diseased okra plant samples, collected from Agriculture Research Institute, Tandojam by PDA plate method.

Multiplication of inoculum: In order to prepare large quantity of *M. phaseolina* inoculum, its sclerotia were obtained by growing the test pathogen on sand+wheat meal substrate. For this purpose, 95 gm of sand and 5 gm of wheat meal were mixed together thoroughly followed by moistened with 10 ml sterilized water. The substrate was transferred into 250 ml conical flask and was sterilized in the autoclave at 15Lbs for 20 minutes. Left it 24 hours for cooling and 5 mm disc from actively growing *M. phaseolina* pure culture was added in the conical flask. Incubation was done at room temperature for 4-6 weeks with continous shaking the flask daily in order to uniform multiplication of the pathogen inoculum. After 4-6 weeks the color of the substrate in the conical flask turned black due to the sclerotial formation. The contents of the conical flash were poured onto the 15µm sieve and black tiny sclerotia present on the surface of the sieve were collected in the sterilized glass beaker for further use Fig.1.

Fig. 1. Scerotia of *Macrophomina phaseolina*.

Fig. 2. Effect of *Macrophomina phaseolina* on growth of okra plants

Effect of different inoculation methods:

Soil Infestation Method: Sterilized soil was artificially infested with the pathogen inoculum at 1/sclerotia gram^{-1} of soil. The thermopol glasses of 7 cm diameter were filled with this soil so that each contained 190 gm soil. Five surface sterilized seeds of okra variety Sabz Pari were sown in 1 cm depth with equal distance to one another in each glass. Non-infested soil served as control. The experiment was designed as Randomized Complete Block Design with four replications. Plants were observed daily and seed germination as well as plant mortality were recorded. After one month of sowing, plants were uprooted and data on plant height, weight and root infection percentage were recorded.

Seed Infestation Method: Seeds of okra were artificially infested with test pathogen by placing them in inoculum suspension of *M. phaseolina*. For this purpose, 10 ml sterilized water was added to the culture plate of *M. phaseolina* and rubbed with finger to detach the fungal growth from the medium. Seeds were soaked into this suspension for 12 hours. The seeds were then dried on the blotter paper and sown in a thermopol glasses each contains 190 gm of sterilized sandy soils at 5 seeds/glass. The un-inoculated seeds served as control. The experiment was carried out as Randomized Complete Block Design with four replications. Plants were observed daily and seed germination as well as plant mortality were recorded. After one month of sowing, plants were uprooted and data on plant height, plant weight and root infection percentage were recorded.

Effect of different inoculum level: Another experiment was conducted to determine the effect of different inoculum density of *M. phaseolina* on plant growth and disease development. For this purpose, already prepared sclerotia of *M. phaseolina* were added in the soil @ of 0 (control), 10, 20, 30, 40 and 50 sclerotia gram^{-1} soil. Thermopol glasses of 7 cm diameter were filled with sterilized soil and five surface sterilized seeds of okra variety Sabz Pari were sown in 1 cm depth with equal distance to one another in each glass. Plants were observed daily and seed germination as well as plant mortality were recorded. After one month of sowing, plants were uprooted and data on plant height, plant weight and root infection percentage were recorded. The root infection percentage was calculated by following formula:

$$Infection\ \% = \frac{No.of\ pieces\ colonized\ by\ pathogen}{Total\ no.of\ pieces\ studies} x\ 100$$

RESULTS

Effect of different inoculation methods: Inoculation of *M. phaseolina* by either method significantly affects the plant growth as well as germination and plant mortality as compared to the un-inoculated (control) plants. It was also evident from the data that soil infestation method was more aggressive for pathogenicity, as it caused significantly more reduction in plant growth as compared to the seed infestation method (Fig. 2). Soil infestation method also significantly ($P<0.05$) affected the germination percent and plant mortality as lowest seed germination (74.5%) and maximum plant mortality (81.19%) was recorded

in plants inoculated by this method, followed by seed infestation method in which germination was 90% and plant mortality was 77.77% (Fig. 3c & 3d). Significantly (P<0.05) maximum root infection was recorded in soil infestation method (79.75%) followed by seed infestation method (69.5%) & control (3.5%) (Fig. 3e).

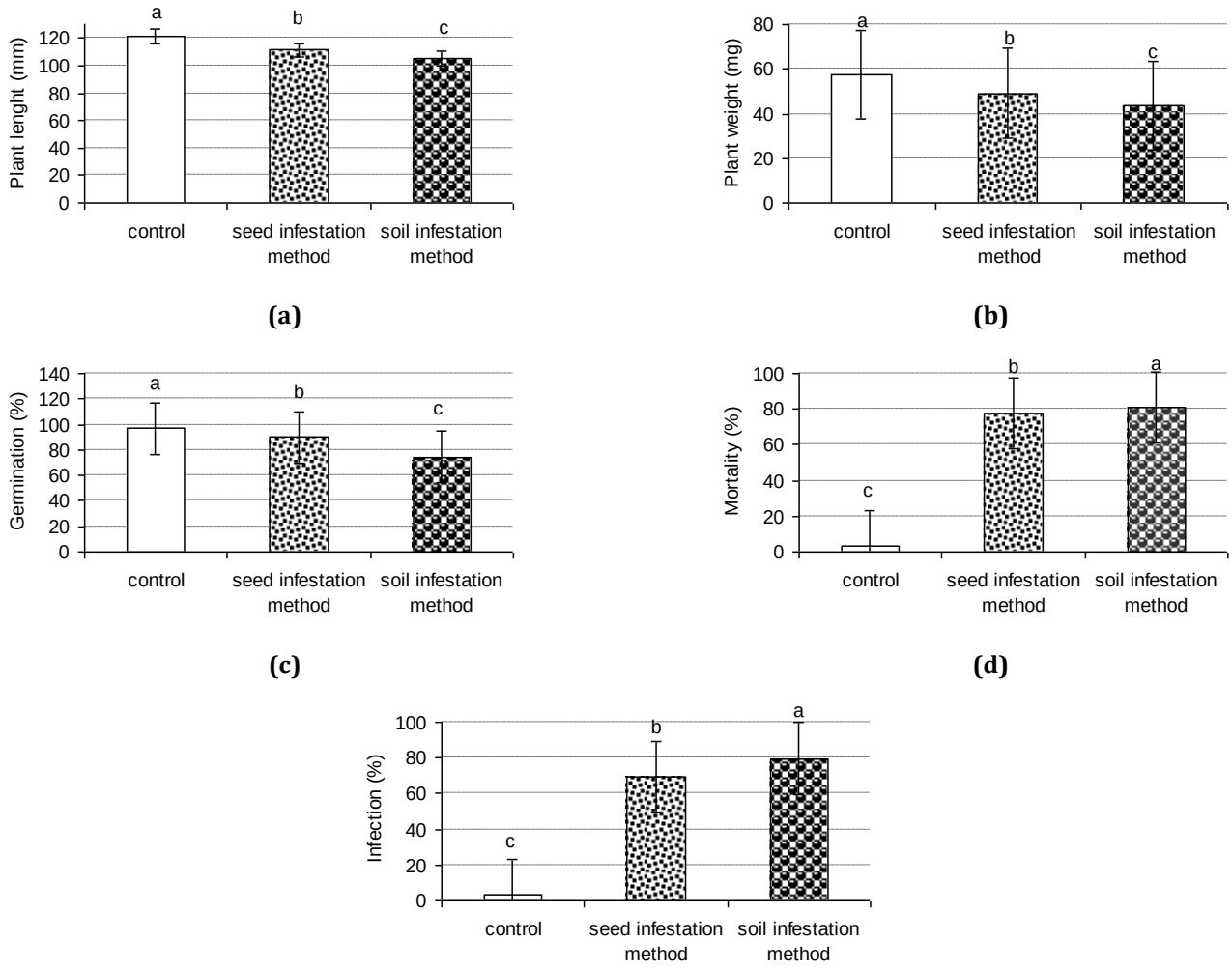

Fig. 3. Effect of *M. phaseolina* on (a) plant length, (b) plant weight, (c) seed germination, (d) plant mortality (e) root infection of okra plants inoculated by seed infestation or soil infestation method.
Means followed by different letters in respective bar are significantly different at P= 0.05.

Effect of different inoculum level: Impact of *M. phaseolina* on inoculated okra plants was increased with increasing inoculum density. Plant length and weight of test plants were gradually decreased with increasing inoculum level applied in the form of sclerotia (Fig. 4a & 4b).

The significantly (P<0.05) minimum plant length and weight (105.16 mm and 43.48 mg) was observed in plants grown in soil inoculated with test pathogen @ 50 sclerotia gram^{-1} soil, whereas control plants (un-inoculated) showed maximum plant length and weight (121.5 mm and 57.64 mg) (Fig. 4a & 4b). Plant mortality and root infection by the *M. phaseolina* is positively correlated with the pathogen population and both were increased with increasing inoculum level (Fig. 4d & 4e). Significant difference (P<0.05) in terms of root infection and plant mortality was observed within different treatments (inoculum levels).

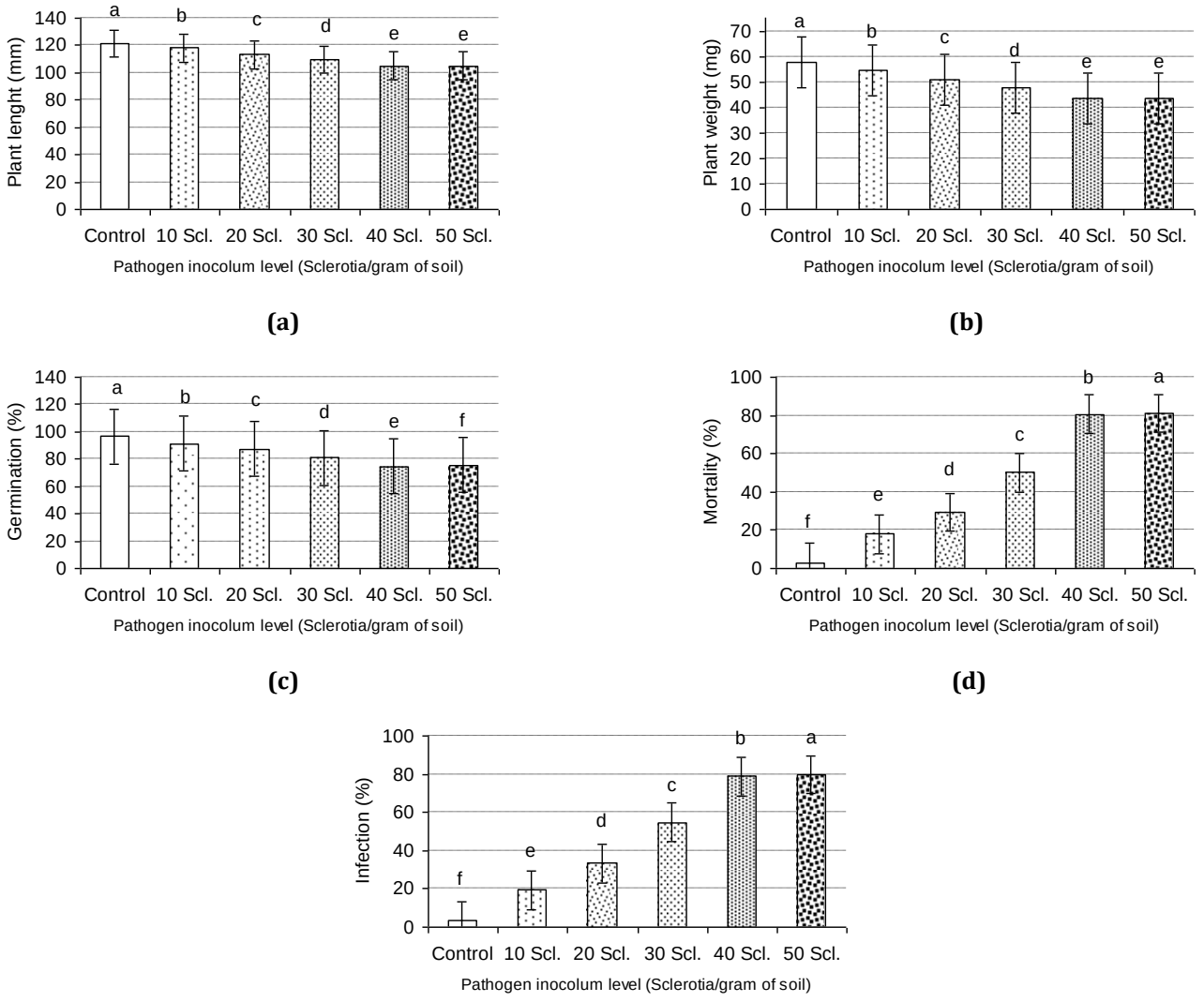

Fig. 4. Effect of different inoculum levels of *M. phaseolina* on (a) plant length, (b) plant weight, (c) seed germination, (d) plant mortality (e) root infection of okra plants.
Means followed by different letters in respective bar are significantly different at P= 0.05.

The highest plant mortality (81.19%) and root infection (79.75%) was recorded in treatments where soil was inoculated with test pathogen @ 50 sclerotia gram^{-1} soil, followed by 40 sclerotia gram^{-1} soil. It was also observed that there was not much difference between inoculum level of 40 and 50 sclerotia in terms of all parameters *viz.*, plant length, plant weight, seed germination, plant mortality and root infection (Fig. 3 & 4).

DISCUSSION

Pathogenicity test, carried out during present study on okra variety Sabz Pari commonly growing in Sindh province, has confirmed that *Macrophomina phaseolina* is an aggressive pathogen of the okra. Its inoculation on test plants significantly reduced seed germination and plant growth while increased the plant mortality. Study also revealed that among two methods evaluated for pathogenicity test, soil infestation method comparatively caused more infection as well as checked much plants growth than seed infestation method.

M. phaseolina is considered as one of the devastating pathogens of the large number of crop plants, in which it caused substantial losses (Shehzad *et al.,* 1988; Khan, 2007). Our findings were in close agreement to those reported by other research worker from elsewhere, such as Agrawal and Singh (2000) concluded that *M. phaseolina* was responsible for die-back and collar rot

diseases, as well as pre- and post-emergence mortality in okra. Dubey and Jha (1999) and Fakir & Mridha (1985) observed that *M. phaseolina* caused die-back, pre- and post-emergence mortality and collar rot diseases in okra. Also, El-Mohamedy (2004) reported that *M. phaseolina* along with *Fusarium solani,* and *Rhizoctonia solani* caused *M. phaseolina* damping-off and root rot diseases in okra.

Similarly, Mashooda *et al*. (2005) also observed that *M. phaseolina* and *Fusarium verticilloides* were responsible for collar rot, seedling rot and other diseases in okra. They also observed that inoculated seed caused reduced seed germination as well as pre- and post-emergence mortality.

The present study also revealed that plant growth, seed germination, plant mortality and root infection of okra plants was adversely affected with the increasing inoculum levels of *M. phaseolina.* Our results in confirmation to those reported by Dawar and Ghaffar (1998), Moradia (2011) and McCain & Scharpf (1989) who found that increasing sclerotial population of *M. phaseolina* increased the infection and colonization in sunflower, groundnut and conifers, respectively. Similarly, Umamaheswari *et al*. (2001) observed that in groundnut root infection was severely increased by increasing inoculum density of *M. phaseolina*. Kenerley and Bruck (I987) also observed greatest increased in total mortality of Fraser fir seedling with increasing inoculum density of *Phytophthora cinnamomi.*

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