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Influence of Inoculation Methods and Inoculum Levels on the Aggressiveness of *Fusarium oxysporum* f.sp. *ciceris* on Chickpea and Plant Growth

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Abstract

To screen the plants against diseases suitable inoculation method together with appropriate inoculum density are essential. Measurements of propagules required for infection are also necessary for the development of potential disease risk analysis. To find out the best method for the pathogenicity test of chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris* five inoculation methods *viz*., soil infestation, seed infestation, drenching, injection and spraying methods were evaluated. The soil infestation, seed infestation followed by drenching method were highly effective in establishing the *F. oxysporum* f.sp. *ciceris* infection in chickpea plants. The plant inoculation with these methods produces higher plant mortality and higher infection as well as reduced plant growth. In order to determine the effective inoculum level of *F. oxysporum* f.sp. *ciceris* for the development of disease, chickpea plants were inoculated with 10^1, 10^2, 10^3, 10^4, 10^5 and10^6 spores g^{-1} of soil and found out that fourth inoculum level (10^4 spores g^{-1} soil) enough to produce the maximum disease determined by plant mortality, root infection and plant growth. The results indicated that up to certain level disease development (plant mortality, pathogen infection and plant growth) positively correlated with the increasing inoculum densities. © 2016 Friends Science Publishers

Keywords: Wilt; Chickpea; Pathogenicity; Inoculum level; *Fusarium*

Introduction

Chickpea (*Cicer arietinum* L.) a bushy annual plant belongs to the Leguminaceae family. The chickpea is popular due to its higher nutritional and medicinal values. Its grain contains 13–33% protein, 40–50% carbohydrates and 4–10% oil (Hulse, 1991; Stallknecht *et al*., 1995; Muehlbauer and Rajesh, 2008). Worldwide it is grown on an area of 13.5 million hectares with a production of more than 13 million tons. Among edible pulses, it ranks second after dry bean. In Pakistan, area under chickpea cultivation is 992 thousand hectares with production of 751 thousand tones. Domestic production is not enough to meet local needs. Therefore, Pakistan imported 0.28 metric tons of chickpea of 184387 thousands US$ in 2011 (FAOSTAT, 2013). Major reasons for the low productivity are disease susceptibility, environmental stress, insect pest infestation, poor crop management and low yielding varieties. Although efforts have been made to check the potential of existing germplasm as well as to develop high yielding varieties with desirable characters (Ali *et al*., 2002; Saleem *et al*., 2002; Ghazanfar *et al*., 2010).

More than 50 pathogens of chickpea have been reported in the world, including fungi, bacteria, nematodes and plant viruses (Nene *et al*., 1996), but few have the highest potential to devastate the crop. Annual yield losses in chickpea have been estimated to be 4.8 million tones worldwide due to biotic stresses, including infectious plant diseases (Ryan, 1997). Among these diseases, Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceris* (Padwick) Matuo and K. Sato, is considered the most serious and widespread throughout the chickpea growing areas of the world (Trapero-Casas and Jimenez-Diaz, 1985; Nene and Reddy, 1987; Haware, 1990; Jalali and Chand, 1992). Under severe conditions, the wilt infection can damage the crop completely and cause 100% yield loss (Halila and Strange, 1996; Navas-Cortes *et al*., 2000a). However, yield losses of 10–15% were reported as a regular feature of this destructive disease (Campbell and Madden, 1990; Haware, 1990). The chickpea wilt fungus *F. oxysporum* f.sp. *ciceris* is vascular pathogen that perpetuates through seed and soil. It can survive in soil, even in the absence of a host for 3–6 years (Haware *et al*., 1996). This pathogen can cause infection at all stages of plant growth with more incidence at flowering and poding stage. Because of wilt infection, the complete plant or plant parts may die within few weeks of infection. In field conditions, the typical wilting can appear within 3–4 weeks after sowing, if the variety is susceptible (Haware, 1990).

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An effective way to deal with disease is the use of resistant variety (Jimenez-Diaz *et al*., 1993). An optimized inoculation method is required to screen the germplasm for disease resistance. Number of inoculation techniques has been reported for *F. oxysporum* f.sp. *ciceris* such as root inoculation by dipping in conidial suspension, seed infestation method (Pande *et al.*, 2007) and soil infestation method (Khan *et al*., 2004). Different methods of pathogen inoculation result in very different level of disease. Moreover, inoculums level is also proportional to the amount of disease produced. The present studies were undertaken to determine the best inoculation methods and the inoculum level of *F. oxysporum* f.sp. *ciceris* require to establish the optimum disease severity in chickpea plants.

Materials and Methods

Isolation of Pathogen

The roots of wilted chickpea plants collected in the month of March-2013 from district Larkana, Sindh, Pakistan. The Fusarium wilt pathogen was isolated by tissue isolation method as described by (Waller *et al*., 1998). The isolated fungus was purified and maintained on PDA medium.

Preparation of Conidial Suspension

For inoculation, conidial suspension was prepared from 7 days old culture of *Fusarium oxysporum* f.sp. *ciceris.* Ten ml of sterilized water was added in culture plate, rubbed with sterilized spatula gently to remove the conidia. Suspension was strained with double layered muslin cloth and collected in a sterilized glass beaker. The conidial density was adjusted to 10^5 conidia/mL with the help of hemocytometer (Waller *et al*., 1998; Yang *et al*., 2011).

Effect of Different Inoculation Methods

Five different methods of inoculation *viz.*, soil infestation method, seed infestation method, drenching method, injection method and spraying method were evaluated for their effectiveness to cause maximum pathogenicity on inoculated plants. The test was conducted in earthen pots of 20 cm diameter. Pots were washed thoroughly with distilled sterilized water, dried and then sprayed with spirit to avoid saprophytic contamination. All pots were filled with 2 kg of steam sterilized sandy clay loam soil. The details of each method are described below.

a) Soil infestation method: The seeds of commonly growing local variety 'Rabbat' were obtained from Pulse Station, Agriculture Research Institute, Tandojam. Steam sterilized soil was artificially infested with pathogen inoculum (10^5 conidia/g of soil) before sowings of seeds. The un-inoculated soil served as control. Chickpea seeds washed with distilled sterilized water, rinsed with 5% commercial bleach (Sodium hypochlorite) for 1–1.5 min, again washed two times with distilled sterilized water and dried thoroughly. Ten sterilized seeds of chickpea were sown in each pot at 1 cm depth. The soil moisture was maintained at 50% WHC. The experiment was designed as Complete Randomized Design (CRD) with three replications. After 45 days of sowing (DAS) plants were uprooted and data regarding plant length, plant weight and root infection percentage were recorded by using the following formula:

$$\text{Root infection (\%)} = \frac{\text{Number of root pieces colonized by the fungus}}{\text{Total number of pieces studied}} \times 100$$

b) Seed infestation method: The seeds of chickpea were artificially infested with test fungus by placing them in 100 mL inoculum suspension (10^5 conidia per milliliter) *Fusarium oxysporum* f.sp. *ciceris.* The seeds were then dried on the blotter paper and sown in earthen pots. The un-inoculated pots served as control. Experimental design and observations taken were the same as described above.

c) Drenching method: The earthen pots filled with steam sterilized soil, 10 surface sterilized seeds per pot were sown. After 7 days of sowing, 10 mL of conidial suspension was drenched in each pot. In un-inoculated pots, 10 mL of distilled sterilized water was drenched in the same manner, and served as control. Experimental design and observations taken were the same as described above.

d) Injection method: Ten surfaced sterilized seeds of chickpea were sown in earthen pots filled with sterilized soil at 2 kg/pot. After 15 days of sowing 20 µL of conidial suspensions was injected in stem of each plant with the help of disposable syringe. Control plants (un-inoculated) were injected with 20 µL of distilled sterilized water. Experimental design and observations taken were the same as described above.

e) Spraying method: The chickpea plants were raised in earthen pots as described above. After 15 days of sowing, each pot was sprayed with 10 mL of conidial suspensions with the help of atomizer. The soil surface of each pot temporarily covered with the polyethylene sheet to avoid the contact of the pathogen inoculum with the soil. The control plants were sprayed with the distilled sterilized water. Experimental design and observations taken were the same as described in soil infestation method.

Re-isolation of the Test Fungus

The test fungus was re-isolated to confirm the Koch's postulates. For this purpose, roots were washed thoroughly with tap water and cut into small pieces. After surface sterilization with 5% bleach solution for 1–2 min, root pieces then placed on PDA plates. Five days after incubation, the recovery of inoculated fungus was recorded and infection percent was calculated with the help of following formula:

$$\text{Infection \%} = \frac{\text{Number of pieces colonized by the fungus}}{\text{Total number of pieces studied}} \times 100$$

Evaluation of different Inoculum Levels on Disease Development

The test was conducted in earthen pots of 20 cm diameter. Pots were sprayed with spirit to avoid any saprophytic contamination and filled with steam sterilized sandy clay loam soil at 2 kg/pot. The prepared inoculum suspension was diluted by adding distilled sterilized water to obtain required inoculum density. The steam sterilized soil artificially infested with conidial suspension of *Fusarium oxysporum* f.sp. *ciceris* at 10^1, 10^2, 10^3, 10^4, 10^5 and10^6 per g of soil. Chickpea seeds were rinsed with 0.01% $HgCl_2$ and washed twice with distilled sterilized water and dried thoroughly on blotter paper. Surface sterilized seeds (10/pot) were sown at 1 cm depth. The un-infested pots served as control. The experiment was designed as Complete Randomized Design (CRD) with three replications. Data on plant mortality, plant growth and root colonization were recorded after 45 days of sowing.

Finally the data were analyzed by ANOVA using Statistix 8.1 software. Least significant differences (LSD) were calculated using significant level at $P = 0.05$.

Results

Effect of Different Inoculation Method

Plant mortality varied greatly in all treatments. Pathogen inoculation by all methods except spraying method caused profound plant mortality (ranging from 6.66–73.33%) in chickpea plants inoculated with *F. oxysporum* f.sp. *ciceris* as compared to the un-inoculated plants (3.33%) (Fig. 1). However, maximum plant mortality was recorded in soil infestation method (73.33%) and seed infestation (63.33%) followed by drenching method (49.0%). The plants inoculated by injection method also caused 26.7% plant mortality (Fig. 1). Spraying method appeared un-effective to cause sufficient level of pathogen infection, as well as plant mortality. There was no significant difference ($P \leq 0.05$) in spraying methods and un-inoculated plants (control).

The method of pathogen inoculation to chickpea plants significantly influenced the overall plant growth. Soil infestation method appeared highly effective, for the establishment of the infection as significantly ($P \leq 0.05$) minimum plant growth was observed, followed by seed infestation and drenching method (Fig. 1). With regards of plant growth, the inoculation methods can grouped into two categories, first-group comprised of soil infestation method, seed infestation method and drenching method, which caused significant reduction in shoot and root length as well as the weight of the inoculated plants as compared to the un-inoculated plants and plants inoculated by other methods (Fig. 1). Minimum shoot length and weight was recorded in plants inoculated with soil infestation method (8.7 cm and 0.93 g) followed by seed infestation method (9.1 cm and 1.16 g). While the second group consisting of injection method and spraying method, which caused very little reduction in plant growth as compared to the control (Fig. 1). The plants inoculated by spraying method produced significantly higher shoot length and weight (11.93 cm and 3.43 g) followed by injection method (10.43 cm and 3.26 g). Similar trends also observed in root length and weight.

It appeared that disease development was greatly dependent and influenced by the methods of inoculation. Among different methods of inoculation, maximum root infection by inoculated pathogen was recorded in plants inoculated by soil infestation method and seed infestation method followed by drenching method. The minimum root infection was observed in plants inoculated by spraying method or injection method (Fig. 1).

Effect of Different Inoculum Levels

Different inoculum levels significantly ($P \leq 0.05$) influenced the disease development (Fig. 2). The minimum disease mortality of 16.66 and 26.66% occurred with the application of lower inoculum levels of 10^1 and 10^2 spore g^{-1} of soil, respectively (Fig. 2). Plant mortality gradually increased with each increasing inoculum and became maximum at 10^5 spore g^{-1} of soil, afterwards there was no increase in plant mortality with additional inoculum level of 10^6 spore g^{-1} of soil. The highest plant mortality of 67 and 70% observed with 10^5 or 10^6 spore g^{-1} of soil, respectively (Fig. 2).

The plant growth was also adversely affected by increasing inoculum levels of *F. oxysporum* f.sp. *ciceris*. Even the initial levels (10^1 to 10^4 spores g^{-1}) developed significantly adverse effects on plant growth; however, maximum impact of the pathogen was developed at higher inoculum level (10^5 or 10^6 spores g^{-1} soil) (Fig. 2).

Minimum shoot length (12.42 cm and 12.47 cm) and weight (1.73 g and 1.76 g) significantly recorded in plants inoculated with 10^5 or 10^6 spores g^{-1} soil as compared with other inoculum levels. There was non-significant difference between plants inoculated with either fifth inoculum level of 105 spores g^{-1} soil or sixth inoculum level of (106 spores g^{-1} soil) in terms of shoot length and weight (Fig. 2).

More or less similar trends also observed in the case of root length and weight. Both significantly reduced to an increasing inoculum level up to the 10^4 spores g^{-1} soil in case of root length and 10^5 spores g^{-1} soil in case of root weight. Afterwards any additional increase in pathogen density failed to either cause further reduction in root length or root weight (Fig. 2).

Root infection by inoculated pathogen (*F. oxysporum* f.sp. *ciceris*) varied with the inoculum levels. The effect of initial inoculum levels were more pronounced than higher inoculum levels. However, after a certain level, the

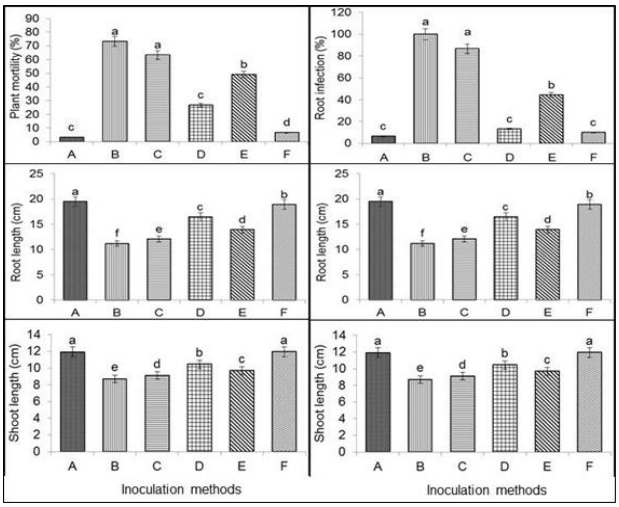

Fig. 1: Effect of different inoculation methods on the plant mortality, root infection and plant growth of chickpea plants inoculated with *F. oxysporum* f.sp. *ciceris.* Bars labeled with the same letter indicating that the mean values are statistically not different according to the Tukey test ($p < 0.05$)

Where: A= Control, B= Soil infestation method, C= Seed infestation method, D=Injection method, E= Drenching method and F= Spraying method

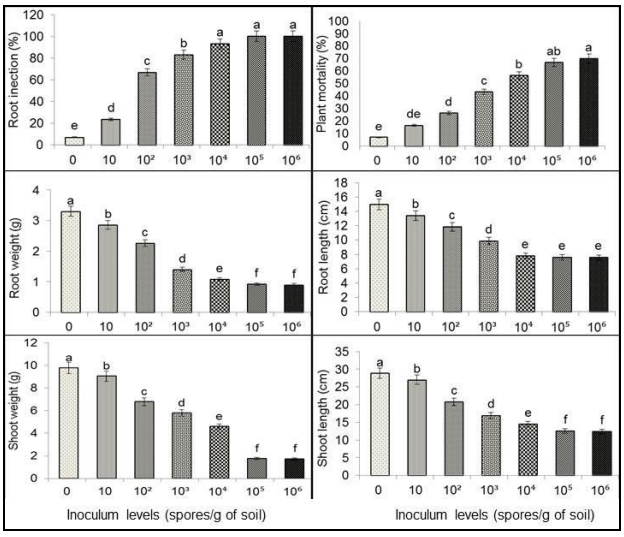

Fig. 2: Effect of different inoculum levels of *F. oxysporum* f.sp. *ciceris plant* mortality, root infection and plant growth of chickpea plants. Bars labeled with the same letter indicating that the mean values are statistically not different according to the Tukey test ($p < 0.05$)

additional pathogen inoculum failed to cause any more impact on root infection. The inoculum level of 10^4 spores g^{-1} soil or above produced maximum root infection. Statistically no significant difference observed between fourth, fifth and sixth inoculum levels. It indicates that the

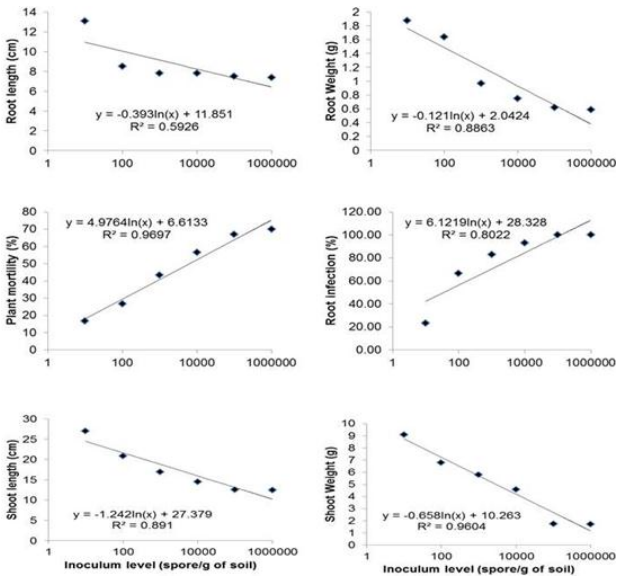

Fig. 3: Regression analysis of root length and weight, plant mortality, root infection, shoot length and weight against different inoculum levels of *F. oxysporum* f.sp. *ciceris*

fourth inoculum level (10^4 spores g^{-1} soil) enough to produce the optimum root infection in chickpea plants inoculated with *F. oxysporum* f.sp. *ciceris*.

Coefficient of determination between inoculums level and plant growth parameter (root length and weight, shoot length and weight), root infection and plant mortality of inoculated plants is ranging from 0.59–0.97, calculated by linear regression equation (Y=mx+b). Correlation results near to one explain the variation in infection, mortality and growth is due to the changing density of inoculums used. The root and shoot length (R^2 = 0.59 and 0.89) as well as root and shoot weight (R^2 =0.89 and 0.96) shows the downhill positive relationship with inoculums level where as plant mortality and root infection (R^2 =0.59 and 0.89) shows strong uphill relationship (Fig. 3).

Discussion

Pathogenicity test confirmed the highly pathogenic nature of the test pathogen. *F. oxysporum* f.sp. *ciceris* causing considerable damage on artificially inoculated chickpea plants (Trapero-Casas and Jiménez-Díaz, 1982). Inoculation method and inoculums level of *F. oxysporum* f.sp. *ciceris* influence the plant mortality and pathogen infection as well as plant growth of chickpea. Among five different inoculation methods for pathogenicity test, the soil infestation and seed infestation methods were highly effective in establishing the *F. oxysporum* f.sp. *ciceris* infection in chickpea plants. The disease was more pronounced in these methods of inoculation as compared to the drenching, spraying and injection methods. Either the plants inoculated through soil infestation, seed infestation or drenching showed

significantly higher plant mortality and pathogen infection as well as reduced plant growth. The soil infestation method and drenching method may efficiently be used in greenhouses for inoculation with wilt pathogens, but have limitation for screening a large number of plants in the field soil. Technically sterilization of field is impossible otherwise there may present antagonistic organisms that may interfere with the normal pathogenicity procedure (Sinclair and Dhingra, 1995). In our study seed infestation methods produce equal plant mortality and infection as compared to soil infestation method, hence may be used for screening a large number of plants. Injection method was difficult due to the small diameter of stem and spraying method was not suitable for vascular wilt pathogen it rather suits for the pathogens causing foliar diseases. Direct introduction of the vascular wilt pathogen to the stem does not allow the activation of resistance mechanism present in the roots for soil borne pathogen getting roots as the main point of entry (Cirulli *et al*., 2008).

Different inoculum levels of *Fusarium oxysporum* f.sp. *ciceris* influenced the disease development. The results indicated that up to certain stage disease development (plant mortality, pathogen infection and plant growth) was positively correlated with the increasing inoculum levels. Significant correlation between number of lesion and shoot weight and between shoot weight and pods reported in kidney bean inoculated with *Rhizoctonia solani*. The impact of pathogen inoculation was more pronounced at initial levels, reached to peak at 4^{th} or 5^{th} inoculum level and then static. It means at the level of 104 or 105 spores g^{-1} of soil, the soil become fully saturated with the pathogen populations, so that any further increased in pathogen inoculum failed to cause more noticeable change in either disease development or on plant growth. Disease establishment and symptom expression were positively correlated with the pathogen inoculum (Yaqub and Shahzad, 2005; Navas-Cortes *et al*., 2007), infection increase in response to increased inoculum density (Tooley *et al*., 2013). As the inoculum density increases, wilt disease symptoms appear earlier in susceptible cultivar, while at low level of inoculum densities, wilt disease of chickpea appears late and develop slowly (Sugha *et al*., 1994; Zote *et al*., 1996; Pande, 2007).

Moreover, the effective inoculum levels to produce disease greatly varied with the pathogen race and variety under evaluation (Navas-Cortes *et al*., 2000b). Similarly Landa *et al*. (2001) observed that at optimum temperature, disease development was greater at 250–1000 chlamydospores g^{-1} of soil as compared to the 25–100 chlamydospores g^{-1} of soil.

References

Ali, S., A.B. Maher, M. Anwar and A.M. Haqqani, 2002. Exploitation of genetic variability for grain yield improvement in chickpea. *Int. J. Agric. Biol*., 4: 148–149

Campbell, C.L. and L.V. Madden, 1990. *Introduction to Plant Disease Epidemiology*. John Wiley and Sons, New York, USA

Cirulli, M., C. Colella, M.D. Amico, M. Amenduni and G. Bubici, 2008. Comparison of screening methods for the evaluation of olive resistance to *Verticillium dahlia* Kleb. *J. Plant Pathol*., 90: 7–14

FAOSTAT, 2013. *Food and Agriculture Organization of the United Nations*. http://faostat3.fao.org/download/Q/QC/E (Accessed: 08 November, 2014)

Ghazanfar, M.U., S.T. Sahi, N. Javed and W. Wakil, 2010. Response of advanced lines of chickpea against chickpea blight disease. *Pak. J. Bot*., 42: 3423–3430

Halila, M.H. and R.N. Strange, 1996. Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f. sp. *ciceris* race. *Phytopathol. Mediterr*., 35: 67–74

Haware, M.P., 1990. Fusarium wilt and other important diseases of chickpea in the Mediterranean area. Options M´editerran´eennes. *S´erie S´eminaires*, 9: 63–166

Haware, M.P., Y.L. Nene and M. Natarajan, 1996. The survival of *F. oxysporum* f.sp. *ciceris* in the soil in the absence of chickpea. *Phytopathol. Mediterr*., 35: 9–12

Hulse, J.H., 1991. Nature, composition and utilization of grain legumes. *In*: *Uses of Tropical Legumes*. pp: 11–27: Proceedings of a Consultants' Meeting, 27–30 March 1989, ICRISAT, Patancheru, India

Jalali, B.L. and H. Chand, 1992. Chickpea wilt. *In*: *Plant Diseases of International Importance*, Vol. I. pp: 429–444. U.S. Singh, A.N. Mukhopadhayay, J. Kumar and H.S. Chaube (eds.). Prentice Hall, Englewood Cliffs, New Jersey, USA

Jimenez-Diaz, R.M., A.A. Hervas and J.C. Trapero-Casas, 1993. Pathogenic variability and host resistance in *Fusarium oxysporum* f.sp. *ciceris*/*Cicer arietinum* pathosystem. Hodowla Rosin. *Aklimatyzacja Nasiennictwo*, 37: 87–94

Khan, M.R., S.M. Khan and F.A. Mohiddin, 2004. Biological control of Fusarium wilt of chickpea through seed treatment with the commercial formulation of *Trichoderma harzianum* and/or *Pseudomonas fluorescens. Phytopathol. Mediterr*., 43: 20–25

Landa, B.B., J.A. Navas-Cortés, A. Hervás and R.M. Jiménez-Díaz, 2001. Influence of temperature and inoculum density of *F. oxysporum* f.sp. *ciceris* on suppression of Fusarium wilt of chickpea by rhizosphere bacteria. *Phytopathology*, 91: 807–816

Muehlbauer, F.J. and P.N. Rajesh, 2008. Chickpea, a common source of protein and starch in the semi-arid tropics. *Genomics Trop. Crop Plant*, 1: 171–186

Navas-Cortes, J.A., B. Hau and R.M. Jimenez-Diaz, 2000a. Yield loss in chickpeas in relation to development of *Fusarium* wilt epidemics. *Phytopathology*, 90: 1269–1278

Navas-Cortes, J.A., A.R. Alcala-Jimenez, B. Hau and R.M. Jimenez-Diaz, 2000b. Influence of inoculum density of races 0 and 5 of *F. oxysporum* f.sp. *ciceris* on development of *Fusarium* wilt in chickpea cultivars. *Eur. J. Pl. Pathol*, 106: 135–146

Navas-Cortés, J.A., B.B. Landa, M.A. Méndez-Rodríguez and R.M. Jiménez-Díaz, 2007. Quantitative modeling of the effects of temperature and inoculum density of *Fusarium oxysporum* f.sp. *ciceris* races 0 and 5 on development of Fusarium wilt in chickpea cultivars. *Phytopathology*, 97: 564–573

Nene, Y.L. and M.V. Reddy, 1987. Chickpea diseases and their control. *In*: *The Chickpea*. pp: 233–270, M.C. Saxena and K.B. Singh (eds.). CABI Publishing, Wallingford, UK

Nene, Y.L., V.K. Sheila and S.B. Sharma, 1996. *A World List of Chickpea and Pigeonopea Pathogens*, 5^{th} edition, ICRISAT, Patancheru, India

Pande, S., J.N. Rao and M. Sharma, 2007. Establishment of the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceris* in the soil through seed transmission. *Plant Pathol. J*., 23: 3-6

Ryan, J.G., 1997. A global perspective on pigeon pea and chickpea sustainable production systems-present status and future potential. *In*: *Recent Advances in Pulses Research*. pp: 1–31. A.P. Asthana and M. Ali (eds.). Indian Society of Pulses Research and Development, Kanpur, India

Saleem, M., K. Shahzad, M. Javid and S. Abdul-Ur-Rauf. 2002. Heritability estimates for grain yield and quality characters in chickpea (*Cicer arietinum*). *Int. J. Agri. Biol*., 4: 275–276

Sinclair, J.B. and O.D. Dhingra, 1995. *Basic Plant Pathology Methods*. CRS Press, Inc. Boca Raton, Florida, USA

Stallknecht, G., K.M. Gilberston, G.R. Carloson, J.L. Eckhoff, G.D. Kushnak, J.R. Sims, M.P. Wescott and D.M. Wichman, 1995. Production of chick peas in Montana. *Agri. Res*., 12: 46–50

Sugha, S.K., S.K. Kapoor and B.M. Singh, 1994. Factors influencing Fusarium wilt of chickpea (*Cicer arietinum* L.). *Ind. J. Mycol. Plant Pathol*., 24: 97–102

Tooley, P.W., M. Browning and R.M. Leighty, 2013. Inoculum density relationships for infection of some Eastern US forest species by *Phytophthora ramorum*. *J. Phytopathol*., 161: 595–603

Trapero-Casas, A. and R.M. Jiménez-Díaz, 1982. Occurrence and etiology of chickpea wilt complex. *Rev. Plant Pathol*., 61: 446

Trapero-Casas, A. and R.M. Jiménez-Díaz, 1985. Wilt and root rot diseases of chickpea in southern Spain. *Phytopathology*, 75: 1146–1151

Waller, J.M., B.J. Ritchie and M. Holderness, 1998. *Plant Clinic Handbook*. CAB International, New York, USA

Yang, X., L. Chen, X. Yong and Q. Shen, 2011. Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against Fusarium wilt of cucumbers. *Biol. Fert. Soils*, 47: 239–248

Yaqub, F. and S. Shahzad, 2005. Pathogenicity of *Sclerotium rolfsii* on different crops and effect of inoculum density on colonization of mungbean and sunflower. *Pak. J. Bot*., 37: 175–180

Zote, K.K., M.P. Haware, S. Jayanthi and J.N. Rao, 1996. Effects of inoculum density of *Fusarium oxysporum* f. sp. *ciceris* Race 1 and Race 2 on chickpea wilt. *Phytopath. Medit*., 35: 43–47

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