



Ecology, Biology and Management of Fusarium Wilt in Chickpea (*Cicer arietinum* L.): A Review

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10.18805/ag.R-2481

ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important legume crop in the world and mostly cultivated during the cool winter season (Nov-Feb) in India. In recent, chickpea production and yield drastically reduced in India due to abnormal monsoonal rainfall distribution and soil conditions has been changed due to several improper management of fields and especially soil borne disease like wilt. On regularly, Fusarium wilt incidence occurring early sown cultivars. The wilt incidence occurred during 18-35 days on ramification stage, so the plant loses their reproduction stage and yield also retarded. Several strategies were applied in management of wilt but the pathogen (*F. oxysporum* f. sp. *ciceris*) recovered their virulence ability and broke the host resistance. Continuously screened the selection of resistant lines, combined application of biocontrol treatments, plant defense activators and fungicides recorded the highest yield with least incidence of wilt.

Key words: Chickpea, Fusarium, Legumes, Plant defense activators, Wilt.

Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world by production (15.4%) after common pea (*Phaseolus vulgaris*) and peas (*Pisum sativum*) belonging to *Leguminosae*. It is the largest cultivated grain legume crop in the *Cicer* genus (Chand and Khirbat, 2009). This plant is a member of the papilionoid subfamily of legumes that originated from its wild *Cicer reticulatus* ancestor in a relatively small area in Turkish Kurdistan 8000 - 9000 years ago (Lev-Yadun *et al.*, 2000). Global wide, chickpea production was decreased by the viable contribution of the soil borne diseases viz., Fusarium wilt, black rot, dry root rot and collar rot. Among them the most potential role was played by the wilt which causes severe yield losses ranging from 24 to 65% in all tropical countries and managed through several strategies viz., cultural, chemical and biological (Jimenez-Diaz *et al.*, 2015). An attempt has been made to review pertinent literature under the following headings.

Crop importance

It was mostly cultivated for easily available plant derived dietary protein which contains vitamins, minerals, fibres and fats (Roy *et al.*, 2010). Chickpea seeds are a major source of human food and animal feed because of their high content of lysine-rich protein (Jukanti *et al.*, 2012). The considerable amount of fat content ranging from 3.8-10.2% (Adarsh *et al.*, 2019). It also improves soil fertility by fixing atmospheric nitrogen into available form in the rhizosphere. In the global level pulses occupied an area, production and productivity of 80.8 million ha, 70.3 MT and 904 kg/ha (Singh *et al.*, 2017). In India, the total area of pulses is 851.9 lakh ha, 774.73 lakh tonnes and productivity of 909 kg/ha (Rajender, 2018). India accounts for 75% of world's chickpea production on 13.98 million ha area with

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How to cite this article: Sankar, P.M., Shreedevasena, S., Karthiba, L., Raju, P.A., Vanitha, S., Kamalakannan, A. and Jeyakumar, P. (2022). Ecology, Biology and Management of Fusarium Wilt in Chickpea (*Cicer arietinum* L.): A Review. *Agricultural Reviews*. DOI: 10.18805/ag.R-2481.

Submitted: 19-01-2022 **Accepted:** 28-05-2022 **Online:** 03-06-2022

production 137.3 lakh tonnes and productivity 982 kg/ha which represents 40 to 68.0% and 48.1% of the national pulse acreage and production (Thaware *et al.*, 2016). In Tamil Nadu, chickpea was cultivated in an area of 6820 hectares with a production of 4177 tonnes and a productivity of 645 kg/ha. There are four major districts where cultivation in Tamil Nadu viz., Tiruppur (2441 ha), Dharmapuri (2110 ha), Coimbatore (892 ha) and Dindigul (537 ha) (Murali Sankar *et al.*, 2018).

Occurrence and distribution of fusarium wilt disease

On a global level, 89.2% is grown in Asia and accounts for 84.5% of the world production. India is the leading chickpea-producing country with 73.3% of the world acreage and 67.4% of the production (Jimenez-Diaz *et al.*, 2015). Chickpea was infected by more than 52 pathogens. Among the pathogens *Fusarium oxysporum* f. sp. *ciceris* plays a potential role in causing wilt and severe yield loss. During favourable conditions it may cause yield losses up to 100% (Pande *et al.*, 2011). *Fusarium oxysporum* f. sp. *ciceris* causes wilt incidence up to 61.0% at vegetative and 43.0% at flowering stage (Nikam *et al.*, 2011). Early stage wilting causes more losses compared to late wilt, the early wilt accounts 77-94% losses, while late wilting causes 24-65%. The heavy loss in seed weight about 90.9% due to the occurrence of wilt from flowering to podding stage (Ullah Khan *et al.*, 2001). Every year, wilt disease causes 10 to 50% yield loss in Pakistan. In tropical regions, *Fusarium* wilt is estimated at yield losses of 10 % (India and Spain), 40% in Tunisia and 17 % in Iran (Karimi *et al.*, 2012).

Symptomatology

Early wilt

The pathogen causes two types of symptoms through two different pathotypes based on the cultivar nature. If the cultivar is susceptible the incidence occurred within 25 days after sowing in the field referred as early wilt (Al-Taae and Al-Jobory, 2013). The early wilt infected plant showed loss of plant vigour, loss of turgidity in leaves, drooping of tips, loss of chlorophylls, followed by the plant becoming chlorosis or yellowing and the seedlings laid down on the ground within a few days. When uprooted the seedlings showed uneven shrinking of the stem above and below the collar region. The roots of the wilted plants do not show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen, If the cultivar is highly susceptible the seedlings has been completely died within 10 days (Zemouli-Benfreha *et al.*, 2014).

Late wilt

Which usually occurs at 6 to 8 weeks after sowing in the field. The infected plants showed typical wilting on one side of the branches *i.e.*, drooping of the petioles and rachis along with leaflets. Drooping appeared initially in the upper part of the plant, but within a day or after these symptoms spread on the entire plant. Gradually all the leaves turned straw yellow coloured. If examined the infected plant showed no external rotting, drying or discolouration of roots. When split open the stem region above collar portion the inner parts like, xylem vessels are turned to dark brownish or black coloured due to formation of cavity between phloem and xylem, xylem and medulla and phloem and cortical parenchyma, as well as anomalous cellular proliferation in the vascular cambium. This, together with formation of optically dense gels and occlusions in xylem vessels and deposition of tyloses (Dubey and Singh, 2004).

Ecology

Rainfall distribution

The environmental factors *viz.*, atmospheric temperature, relative humidity, soil temperature, soil moisture and rainfall were played a vital role in prevalence of *Fusarium* wilt in chickpea (Merkuz and Getachew, 2012). An erratic and intermittent distribution of rainfall induced sudden increase of the high soil moisture and modified the soil topography, pH and soil organic matter status also (Miller *et al.*, 2003). It's more favourable for incidence of wilt and collar rot ranged from 25 to 48%, normally chickpea required 278 mm enough for their cultivation period (Sharma, 2016).

Soil conditions

F. oxysporum f. sp. *ciceris* can survive in most soil-arctic, tropical, desert, cultivated and non-cultivated. Though *F. oxysporum* may be found in many places and environments, development of the disease is favoured by high temperatures and warm moist soils. The optimum temperature for growth on artificial media is between 25-30°C and the optimum soil temperature for root infection is 30°C or above (Mina and Dubey, 2010). *Fusarium* wilt is most serious during hot weather, when soil temperature ranges from 25 to 32°C with an optimum at about 27°C (Saremi *et al.*, 1999). The first symptoms generally appear about the time of bloom or may occur at any time during the life of the plant (Chand and Kirbhat, 2009). Normally the wilt incidence severely during at 25 to 30°C but not at 15 and 20°C with an inoculum density of 500 and 1000 propagules g⁻¹ soil. No disease developed at 10°C even with an inoculum density of 5000 propagules g⁻¹ soil (Landa *et al.*, 2001). Moderate temperature with low precipitation in long season predisposing yield loss and favour to biotic factors in chickpea (Jumrani and Bhatia, 2014). *F. oxysporum* f. sp. *ciceris* survive and colonize in the acidic pH 5.5 to alkaline pH >8.0 (Khilare and Rafi, 2012).

Soil temperature and moisture

Soil temperature and moisture act as catalysed roles in wilt incidence on chickpea. The growth and survival of *Fusarium* wilt is optimum at 28°C and inhibited above 32°C and not favoured below 17°C (Jimenez-Diaz *et al.*, 2015). Different pathotypes and races were adopted and their incidence ranges with severity is varied through atmospheric and soil temperature (Navas-Cortes *et al.*, 2007). Chickpea cultivar Ayala was moderately resistant [MR] to *F. oxysporum* f. sp. *ciceris* when grown in temperature regime of 21-24°C, but highly susceptible at a temperature regime of 25-27°C by race 1A in cultivars of Ayala and PV-1 (Landa *et al.*, 2006).

Biology of *F. oxysporum* f. sp. *ciceris*

F. oxysporum f. sp. *ciceris* is a ubiquitous soil saprophytic fungus that infects a wide host range of plant species around the world. It can survive in the soil-like resting structure of chlamydospore upto six years. When the crop is available it produces mycelium and penetrates the root cortex of the plant and causes infection and survival (Bennett, 2012). It is a highly

variable pathogen and has a wide host range but *F. oxysporum* f. sp. *ciceris* only pathogenic on *Cicer* spp. crops (Jimenez-Gasco *et al.*, 2002). Mycelial growth of *F. oxysporum* f. sp. *ciceris* produces fluffy, submerged to aerial growth, fungal pigmentation from normal white to pale cream and bicelled numerous microconidia, 3-5 septate sickle shaped macroconidia and terminal or intercalary formation of cylindrical shaped chlamydospores (Nath *et al.*, 2017).

The pathogen exhibits and is distinguished by their two types of pathogenic *viz.*, yellowing and wilting. The yellowing type is characterized through slow progressive leaf yellowing and late death of the plant, while the wilting type is fast and severe chlorosis, flaccidity and early death of the whole plant (Dubey *et al.*, 2010b). In genome of *F. oxysporum* is ranging from 18.1 to 51.5 Mb. The ITS-regions characterization of *F. oxysporum* f. sp. *ciceris* produced a fragment size of 540 bp (Dubey *et al.*, 2010a). Especially ITS-Fu-f and ITS-Fu-r primers and derived an amplicon size of 400 bp on *F. oxysporum* f. sp. *ciceris* (Durai *et al.*, 2012). The *Foc*-gene specific markers derived an amplicon size of 1.5 kb and confirmed as *F. oxysporum* f. sp. *ciceris* (Rakhonde *et al.*, 2015).

Race specifications

In global level totally eight races were reported *viz.*, (1A, 2, 3 and 4) from India, while races (0, 1B/C, 5 and 6) were reported from Mediterranean region and USA for *F. oxysporum* f. sp. *ciceris* (Jimenez-Gasco *et al.*, 2004). The races distinguished by pathogenesis related fourteen pairs of gene specific primers *viz.*, isocitrate lyase, transcription factor, sucrose non-fermenting protein, serine/threonine kinase, chitin binding protein, global nitrogen regulator, cutinase, xylanase 3 gene, kevitone hydratase, trehalose phosphate synthase, MAPK, transposon and desaturase were mostly presented on several species of *Fusarium*. Among these only five GSOs were analysed, Xyl 3 and cutinase gene yielded amplicon 700 bp and 900 bp confirmed as races 1, 2 and 4 of *F. oxysporum* f. sp. *ciceris*. Desaturase gene produced an amplicon size of 600 bp and identified as race 3 (*F. proliferatum*). Amplicon size of 1 kb was produced by the gene of transcription factor for *F. oxysporum* f. sp. *ciceris* and confirmed as all races *viz.*, 1, 2, 3 and 4. the diversity among the races of 1, 2, 3 and 4 through 80 ISSR markers, all UBC series markers were distinguished races 1, 2 and 4 (*F. oxysporum* f. sp. *ciceris*) from race 3 (*F. proliferatum*). Ten primers yielded polymorphic bands between race 1 and 4. Only four primers, namely UBC 834, 835, 868 and 881 were polymorphic for races 1 and 2 (Gurjar *et al.*, 2009).

Five races *viz.*, 1A, 2, 3, 4 and 5 of the *F. oxysporum* f. sp. *ciceris* was governed by a single gene on cultivar WR-315. Based on protein discrimination of races 1, 2, 3 and 4; the whole protein and amino acid profiling is different among them (Desai *et al.*, 1992). Races 0, 1B/C, 4 and 5 were highly virulent against sets of chickpea differential lines *viz.*, Annigeri, ICC4475, CHAPP2 and C-104 (Al-Taae and Al-Jobory, 2013).

Wilt management

Adjusting the time of sowing

Early time of sowing (10th-October) caused severe yield loss (6.34 q/ha) with maximum disease incidence of 32-34%. However, the late sowing (9th-November) attained the least wilt incidence with high germination rate and maximum yield (10.1 q/ha) in chickpea (Andrabi *et al.*, 2011). Third week of November recorded the lowest wilt incidence from 4.5 to 18.1% and maximum yield 820 kg/ha to 1230 kg/ha on cv. GG-2 (Amalraj *et al.*, 2012). Sowing on chickpea cvs. BGD 1005 and Pusa 212 between on early winter to late winter (10th-Nov to 10th-Dec) significantly recorded the least wilt incidence (22.6 to 25.5%) and maximum yield (14.3 to 16.2 t/ha) due to ambient atmospheric temperature (24 to 25.4°C), lowest soil temperature (20.5°C) and > 80% relative humidity (Mina and Dubey, 2010). Chickpea sowing on (18th to 25th-January) recorded least wilt incidence than later sowing of (2nd to 20th-March) on chickpea cultivars Ayala and PV-1 (Landa *et al.*, 2006).

Selection of resistant lines

The use of resistant cultivars for management of wilt is the best and cheapest progressive method in adoptable conditions. This was mostly obtained from varietal screening under *in vivo* conditions. Out of 7000 lines, fourteen lines *viz.*, (P-165, P-289, P-517, P-678, P-1265, P-1270, P-1353, P-4116-1, P-6099, JG-74, NEC-790, WR-315, CPS-1 and BG-212) characterized resistant lines through sick pot and sick plot conditions (Iftikhar *et al.*, 2002). One hundred and ninety-six chickpea lines were screened under sick plot conditions; seven lines *viz.*, (03001, 03006, 03009, 03012, 03016, 03020 and 03045) were identified as highly resistant (Chaudhry *et al.*, 2007). Ten chickpea differential lines for wilt reaction, the cultivars like, JG-62, CPS-1, Annigeri and Chaffa exhibited susceptible reaction to all isolates, whereas L-550 and C-104 were resistant to two isolates (I5 and I8). The cultivars JG-74 and WR-315 showed resistant or moderately resistant against 20 virulent isolates from India (Mandhare *et al.*, 2011).

Biological control

Biocontrol is a potential alternative for fungicidal management against several phytopathogens of economically viable crops. Plant Growth Promoting Rhizobacteria (PGPR) group of *Pseudomonas* spp., *Azospirillum*, *Azotobacter* (Ahmad *et al.*, 2008), *Bacillus* spp. (Cakmakci *et al.*, 2007), *Serratia* spp. (Gyaneshwar *et al.*, 2001), *Burkholderia* (Govindarajan *et al.*, 2006), *Klebsiella* (Govindarajan *et al.*, 2007) and *Beijerinckia* (Thuler *et al.*, 2003). Numerous modes of action have been postulated and demonstrated for antagonistic effects of PGPR in controlling soil borne diseases. Four isolates rhizospheric bacteria *viz.*, *P. putida* (PDBCAB 19), *P. fluorescens* (PDBCAB 2, PDBCAB 29 and PDBCAB 30) were applied as talc formulation through seed treatment, isolates (PDBCAB 19 and PDBCAB 30) completely controlled the wilt in chickpea on field conditions (Rangeshwaran *et al.*, 2000).

Bio-consortia formulation of (PGPR + *Mesorhizobia*) increased the yield and disease reduction in chickpea by application of seed treatment (Kumari and Khanna, 2014). Different species of PGPR viz., (*E. coli* + *P. fluorescens* + *Burkholderia* spp.) were applied through seed treatment and soil application in combined formulation as resulted in better productivity in chickpea (Dasgupta *et al.*, 2015). Seed biopriming (soaking seeds in 10-hrs prior sowing in the talc-based suspension 1% (2×10^8 cfu/g) or 50.0 g formulation / 250 ml of water / kg of seeds with *T. viride*, *T. harzianum* and *T. hamatum* (or) before sowing of soil application of bioagents with FYM was better management for wilt and root rot diseases in chickpea (Pandey *et al.*, 2017).

Plant defense activators

Behaviour of plant pathogen's viz., morphological, molecularly, physiological and biochemical exploration of virulence capability, prevalence and survival potential is often changed due to chemical fungicides, cultivars, abiotic factors and choice of crops also (War *et al.*, 2011). So, an alternative tool is essential for management of plant pathogens infection by their induction of resistance through triggering the R genes expression in plants by application of synthetic chemical compounds (Cohen *et al.*, 2014).

Several organic and inorganic compounds viz., salicylic acid (SA), azibenzolar-S-methyl (BION), 2,6-dichloroisonicotinic acid (INA), β -aminobutyric acid (BABA), probenazole, riboflavin, prohexadione-Ca, Humic acid, KOH, potassium phosphonate and methyl jasmonate (MeJA) directly induced the PR proteins (Sreeja, 2014). Application of salicylic acid (SA) at 1.0 mM reduced the disease incidence of wilt (49.5%) in the susceptible cv. C-727 of chickpea (Chaudhry *et al.*, 2001). Exogenous application of salicylic acid through root dipping method at conc. of 80 μ g /ml reduced the wilt severity (43%) in chickpea under pot conditions (Saikia *et al.*, 2006). Induction of resistance was maximum at 1.0 mM concentration from 5 to 7 days after application and gradually decreased on cv. JG-62 by riboflavin (Sarwar *et al.*, 2003).

Fungicides

Chemical fungicides like Carbendazim (Bavistin) and combination of Carbendazim + Mancozeb (SAFF) through seed treatment at 0.1% recorded 100% wilt reduction in chickpea under field conditions (Mohan Kumar *et al.*, 2017). Soil drenching with Carbendazim- 12% + Mancozeb-63% (SAFF) @ 30DAS reduced the wilt incidence 73.2% in pot culture conditions (Golakiya *et al.*, 2018). Combined application of Carbendazim (Bavistin) + *T. viride* reduced the wilt incidence and increased the pod numbers and individual seed weight also in glasshouse and field conditions (Kumar and Mane, 2017). Dual treatments viz., (seed + soil drenching) with Carbendazim (Bavistin) @ 0.1 and 0.2%, Tetramethyl dithio disulfide (Thiram), Carbendazim + Mancozeb (SAFF), Copper oxychloride (Blue copper) were highly prevented the wilt disease incidence

and reduced seedling mortality of chickpea in glasshouse and field conditions (Maitlo *et al.*, 2014).

CONCLUSION

Fusarium wilt in chickpea was a major threat in chickpea production globally, the pathogen *F. oxysporum* f. sp. *ciceris* is a highly variable nature of growth, colonization and infection also. So, it regularly overcomes the host (chickpea) resistance and causes highly yield loss with their pathogenic nature. The individual management strategy was not applicable to control the pathogen and yield loss. Despite a combined application strategy was given better management in the wilt of chickpea especially on *Rabi* season.

ACKNOWLEDGEMENT

We wish thank to Department of Plant Pathology and Pulses, CPPS and CPBG, TNAU, Coimbatore, India.

Conflict of interest: None.

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