Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6726

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (4):** 696-708 (2018)

International Journal of Pure & Applied Bioscience

Review Article

OPEN ACCESS

Management of Wilt Disease of Pulses: A Review

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Received: 17.07.2018 | Revised: 23.08.2018 | Accepted: 28.08.2018

ABSTRACT

Pulses are important source of protein, vitamin and minerals. They are rich in macro and micronutrients and are useful in sustainable crop system. They add to the nutritional security of the world. They are the best source of irradiating protein malnutrition. The major pulse grown countries are China, India, United States, Brazil, Australia, Canada, Ethiopia, Argentina, Myanmar and Mexico. India is the world largest producer and consumer of pulses. India is the world's largest producer and consumer of pulses. They are grown in three seasons. Legumes are prone to several phytopathological diseases which limit the crop yield, reduce the quality of the produce, ultimately affecting the economy of the region. The pulses infected by approximately 100 fungal diseases all around the world. Among the fungal diseases vascular wilt caused by Fusarium is a major threat to the pulses grower, it is a seed and soil born disease. This review house the information on Fusarium, its species oxysporum and their formae speciales and strains parasitizing chickpea, pigeon pea, pea and lentil with pathogen taxonomy and nomenclature, history, distribution, morphology and culture characteristic, symptomatology, losses and management practices through agronomic practices, chemicals, plant extracts, biological agents, bio fumigations and nanotechnology applications.

Key words**:** *Pulses, Wilt, Fusarium Oxysporum.*

INTRODUCTION

Pulses popularly known as "Poor man's meat" and "Rich man's vegetable are the important sources of proteins, vitamins and minerals which contribute significantly towards the nutritional security of the world[116]. In addition, pulses offer the most practical means of eradicating protein malnutrition, especially among children and nursing mothers. Pulses are grown in most of the countries the major being China, India, United States, Brazil, Australia, Canada, Ethiopia, Argentina, Myanmar, Mexico. In 2011-2013 (average), global pulse production accounted 73.3 million metric tonns[52], and in India production is around 19.3 million tonns[40, 120]. India is the world's largest producers and consumers of pulses. In Indian subcontinent pulses are grown in all three seasons *viz.* i. Kharif, covering *Cajanus cajan* (L.) Millsp, Arhar (Tur); *Vigna mungo* (L.) Hepper, Urd (Blackgram); *Vigna radiate* (L.) Wilezek, Moong (Green gram); *Vigna unguiculate* (L.)

Cite this article: Sinha, P., Rizvi, G. and Parashar, R., Management of Wilt Disease of Pulses: A Review, *Int. J. Pure App. Biosci.* **6(4):** 696-708 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6726

Walp, Lobia (Cowpea); *Macrotyloma uniflorum* (Lam.) Verdc., Kulthi (Horsegram) and *Vigna acontifolia* (Jacq.) Marechal., Moth; ii. Rabi – *Cicer arietinum* (L.), Gram; *Lens culinaris* Medik, Lentil; *Pisum sativum* (L.), Pea, and *Phaseolus vulgaris* (L.), Rajmash iii. Summer – *Vigna radiate* (L.) Wilezek, Greengram; *Vigna mungo* (L.) Hepper, Blackgram and *Vigna unguiculate* (L.) Walp, Cowpea.

The yield and quality of these crops are adversely affected due to diseases amounting around 100 per cent yield losses[28] in certain crop. Diseases are the important factor that frequently limit yields and reduce quality of edible legumes. Although legumes crops are prone to many insect, pests and seed borne disease a major cause of concern as its incidence, if not controlled, devastates the crop. Chickpea, pigeon pea, pea and lentil are attacked by several diseases some of which cause considerable crop damage. Chickpea is mainly affected by wilt (*Fusarium oxysporum* f.sp. *ciceris* Matuo and Sato), blight (*Mycosphaerella pinodes* B. and Blox), rust (*Uromyces ciceris-arietinii* (Grogn.) Jacz. and Boy.). Pigeon pea is affected by wilt (*Fusarium oxysporum* f. sp. *udum* (Butler) Snyd. and Hans.), and sterility mosaic. Pea is affected by Powdery mildew (*Erysiphe polygoni* DC) Wilt (*Fusarium oxysporum* f.sp. *pisi*) and rust (*Uromyces vicia-fabae* (pers.) Schroet.). Lentil is affected by Rust (*Uromyces viciae-fabae* [Pers.] J. Schrot), collar rot (*Sclerotium rolfsii Athelia rolfsii [teleomorph] = Corticium rolfsii*) and wilt (*Fusarium oxysporum* f.sp. *lentis*). Among these disease, *Fusarium* wilt is wide spread in legumes growing regions.

The morphological and cultural characters of *Fusarium* are similar, among isolates, which cause wilt of pulses, the fungus is host specific so called as *formae speciales*. Each *formae speciales* produce different symptoms. *Fusarium oxysporum* Schlecht and Emnd Snyd. & Hans. f.sp. *ciceri* (Padwick) Snyd. & Hans. affects chickpea, *Fusarium oxysporum* f.sp. *udum* Butler affects pigeon pea, *Fusarium oxysporum* Schl. f.sp. *pisi* (van Hall) Snyd. & Hans. affects pea and *Fusarium oxysporum* f.sp. *lentis* (Vasudeva and Srinivasan) affect lentil.

PATHOGEN

Fusarium is a cosmopolitan fungus that is found in soil from parts of the world. It is found not only in the temperate and tropical areas of the world but also occurs in such diverse environment as the arctic and deserts[42,87]. On plants, *Fusarium* causes cortical rots, head blights, leaf spots, root rots, fruit rots, cankers, dieback and vascular wilt diseases. Of all the diseases caused by *Fusarium*, vascular wilt is the most important disease caused by formae speciales of *Fusarium oxysporum*[85,7]. Many plants have at least one *Fusarium*-associated disease. A recent perusal of the plant disease list maintained by the American Phytopathological Society(www.apsnet.org/online/common/search.asp) revealed that over 81 of the 101 economically important plants on the list had at least one associated *Fusarium* disease[66].

TAXONOMY AND NOMENCLATURE

Fusarium belongs to class Ascomycetes, order Hypocreales, genus Fusarium[67]. Genus *Fusarium* was erected by Link in 1809 for the species with fusiform, non-septate spores borne on a stroma[20,105]. Approximately 1000 species had been described during the period 1809-1935. In 1935 Wollenweber & Reinking (Germany), organized the genus in 16 sections, 65 species, 55 varieties, 22 forms and Gerlach (1982), mentioned 78 species. Snyder& Hansen (1940s), USA compressed the 16 sections into nine species, and the species in section Elegans into a single species, F. oxysporum and Nelson, Toussoun & Massas [87], mentioned 30 species. In Russia, Bilai (1955) and Railo (1959) mentioned 9 sections, 26 species, 29 varieties and 55 species respectively. Gordon[125] mentioned 26 species at Canada. Messiaen & Cassi (1968), France mentioned 9 species. Booth[20, 21, 22], England 48 species and 104 forms and varieties. Mato[91], Japan 10 species. Joffe (1974), Israel 13 sections,33 species,14 varieties. Leslie and Summerell[66] summarized information for 70 species of Fusarium[110]. Historically strains of

F. *oxysporum* have been divided into *formae speciales* on the basis of virulence on a particular host or group of hosts[33]. At species level characters, like shape of macroconidia and presence or absence of microconidia etc. are considered for comparison and taxonomic purpose[121].

HISTORICAL

At the beginning of nineteenth century, this devastating fungus under the genus Fusarium was first reported in pigeon pea crop by E. J. Butler in India. The name *Fusarium udum* was accepted as an imperfect state[21] because of the macro-conidia having well distinguished prominent hook. *F. udum* is host specific to pigeon pea[92,122,21,97]. In 1918 Butler discovered another formae speciales of F. *oxysporum* which causes wilt disease in chickpea (*Cicer arietinum* L.) and named *Fusarium oxysporum* f.sp. *ciceri.* McRae[74] as well as Prasad and Padwick[101] reported *F. oxysporum* f.sp. *ciceris* pathogenic to chickpea crop which is now accepted all over the world as the causal agent of *cicer* spp.[22,53,68]. The Fusarium wilt of pea was first recognized during 1918 by Bisby in Minnesota[31,76]. Prissyajnyak first reported lentil wilt caused by *Fusarium oxysporum* f.sp. *lentis* from Russia (U.S.S.R.) in 1931. After three years, the occurrence of such a disease was reported from undivided Bengal, India[6,7].

DISTRIBUTION:

It is an important fungal disease in all pulses growing region around the world. Chickpea wilt widely occurred in Spain[51], Syria[48], India[5], Pakistan[45], Ethiopia[75], Mexico[8], Nebraska, USA[47], Iran[77], and Sudan[3] where ever gram (*Cicer arietinum* L.) is grown[68]. Wilt disease in pigeon pea has been reported from 15 countries[88]. Although the disease is more prevalent in India, East Africa and Malawi, it also occurs in Bangladesh, Grenada**,** Indonesia, Mauritius, Myanmar, Nepal, Nevis, Venezuela, Trinidad, and Tobago[55,107,73]. Recently, this pathogen was reported to be spreading in Southern Africa reaching areas in Mozambique (Southern Zambezian province)[43,19], but it is apparently more important in India and Eastern Africa[62]. Pea wilt reported to occur worldwide in the countries *viz.,* India, Bangladesh, Brazil, Philippines, South Australia, Taiwan, Thailand, Tropical Africa, France, USA, Pakistan, China, Rassia, Canada and many other countries[64]. Lentil wilt is widespread in most of the lentil growing countries of the world, particular in America[131], Bangladesh[12], Argentina[106], Brazil[126], India[125], Italy[123], France[78], Czechoslovakia[124], Myanmar and Pakistan[11], Nepal[56], Syria[15,39], Uruguay[24], and USSR[63,7].

MORPHOLOGY AND CULTURAL CHARACTERS:

The fungus is known to produced sparse to abundant aerial mycelium, and white, pink, salmon and purple pigmentation on the reverse side of the colony in culture[42,87,65]. *F. oxysporum* is an asexual fungus that produce three types of spores- microconidia, macroconidia and chlamydospores[87]. Morphological characterization (Table 1) of *F. oxysporum* is based on the shape of macroconidia, the structure of microconidiophores and the formation and disposition of clamydospores[16]. The optimum growth of *F. oxysporum* was found to be between 25^0- 28^0C. Cook and Bekar in their review of the biological control of plant diseases noted that the growth of the *Fusarium* wilt pathogen is generally maximal at 28^0C, inhibited above 33^0C and not favored below 17^0C. The optimum p^H for growth of the F. *oxysporum* ranged from 6.5-7.0[58]. Optimum radial growth and maximum sporulation was found at 6.5 P^H, followed by p^H 7.5[18]. Effect of p^H on the germination of chlamydospores of *F. Oxysporum* was observed in different experiments conducted over a wide range of p^H. The optimum p^H for the growth of F. *Oxysporum* was 5.5[30,100,65].

SYMPTOMATOLOGY:

F. *oxysporum*, produces symptoms such as wilting, chlorosis, necrosis, pre-mature leaf drop, browning of the vascular system, stunting, damping off. At stage, the fungus attacks the host vascular system by entering through wounded root tips. In early seedling stage symptoms do not appear later, symptoms are clearly visible. The initial visible symptoms are interveinal clearing and loss of turgidity in leaves[109,49,19]. In chickpea, the symptoms are yellowing, drooping, drying of the leaves and discoloration of vascular system[69]. The *F. oxysporum* remain as mycelium and chlamydospores in seed and also in the soil over 6 years[115,68]. Pathogenic form of *F. oxysporum* are reported to penetrate a host root either through wound or directly through root apices[86]. In pigeon pea wilt the symptoms usually appear when the crop is in flowering or podding stage, occasionally may be seen at seedling stage. The most characteristic symptoms are browning or blackening of the xylem vessels and a purple band extending upwards from the base of the main stem. The affected young plants (1-2 months) die from wilt, they may not show morphological[4,19]. The most common point of entry by the *Fusarium* wilt pathogen into the pea plant are the root tips, the cotyledonary node, or wounded roots[130,129,36,91]. The lentil wilt pathogen affects the plant at three stages[125,32,60] i.e. pre-emergence stage, post-emergence stage and adult stage. Under early infection, pre-and early post-emergence mortality is noticed. In advance stage, walls of xylem vessels discoloured, brown and contain fungal hyphae. Infected roots of some young plants produce brownish red lesions. Affected old plants turned yellow, droop and wither[7]. In advanced stage of disease development, the wilt fungus grows out of the vascular system into the adjacent parenchyma producing conidia and chlamydospores.

LOSSES:

Wilt diseases caused by *Fusarium* spp. are an important factor that limit yields and reduce quality of edible legumes. The yield losses amount around 50 per cent in pulse crop. In chickpea crop, under favorable condition damage due to wilt disease cause 100% yield loss[83,44,99]. In pigeon pea, wilt is predominant in all major pigeon pea growing areas throughout the world and causes 30-100% yield loss[19]. In pea the disease can cause 25-50 % yield loss [89]. In lentil, the extent of the damage to the crop due to the disease ranges from 20-24% annually[111,2,50].

MANAGEMENT OF WILT DISEASE:

Several management practices (agronomic, chemical, biological and use of resistant varieties) have been adopted to minimize the diseases. Among these control measures, chemical and biological control are more important[70]. There is no doubt that chemical is the best method for management of the disease. Considering adverse effect of chemical on human health and environment, any alternate of chemical fungicide such as biocontrol should be practiced and promoted. Disease management with biocontrol agents offers a great promise[104,119]. These agents are vital component of sustainable agriculture[132], which colonize the rhizosphere (the site requiring protection) and leave no toxic residues as chemicals[37,35].

AGRONOMIC PRACTICES:

Early planted crops are usually affected more with disease. Several studies suggested that delay planting and low temperature during showing is positively related to disease control[26]. The studies of Navas-Cortes *et al.*[82] showed that for every year of experiment epidemic development was related to the date of sowing. Plants spaced at 15-20 cm had much greater disease incidence than those spaced at 7.5 cm; this was due to the shallow root system in widely spaced plants which were susceptible to wilt disease when subjected to moisture stress[10]. Planting of seeds at proper depth (10-12cm) can reduce the disease incidence[118], while shallow sown crop can be affected by disease[34]. Agrawal *et al.*[1] noted effect of wheat, barley, linseed and mustard intercrops/mixed cropping with chickpea on wilt incidence. Intercropping/mixed cropping reduced wilt incidence and increased yield of chickpea. Lowest wilt incidence obtained with intercropping and mixed cropping with linseed[68]. Intercropping with sorghum produced a large reduction in wilt incidence in pigeon pea in the first year (down to 55%) and thereafter it stabilised at about 20–30%[81]. Mixed cropping of chickpea with wheat and berseem gives measurable disease control[46,13].

CHEMICAL CONTROL METHOD:

Since the first use of fungicides in the 1800s, synthetic chemicals have provided much needed relief in the management of plant fungal disease in agricultural production. Seed treatment method with some chemicals such as equivalent mixture of benomyl and thiram[108], a combination of carbendazim + thiophanate (0.15 + 0.10%)[72] were found to be very effective in dropping the Fusarium wilt population. It was also reported that application of boron (Bo), zinc (Zn) or manganese (Mn) and methyl bromide (CH_3Br) to the soil drastically reduce the disease event of Fusarium wilt[71,19]. Lentil wilt is controlled by the seed treatment with thiram + pentachloronitrobenzene or thiram + carboxin[14,67]. Antracol, Captan, Benlate, Topsin-M, Cobox, Dithane M-45, Acrobat, Pentachloronitro- benzene (PCNB), Ridomil, Sancozeb, Vitavax, Daconil and Trimiltox-forte are sensitive against *oxysporum* f. sp. *ciceris* and studied on the basis of fungicides sensitivity @ 100 ppm using poison food technique (68). Raxil Ultra (Tebuconazole), Topsin-M (Thiophanate Methyl), Score (Difenconazole), Derosil (Carbendazim), Hombre (Imidacloprid+Tebuconazole) and Divident Star (MetalaxylM+Difenconazole) show fungicidal property against pea wilt[59]. Fungicides are frequently toxic to non-target organisms like earthworms, microbes and humans (genotoxicity) causing imbalances in the ecosystems[84,96,114].

CONTROL THROUGH PLANT EXTRACTS

The antifungal effect of aqueous extracts of four plant species viz; *Azadaracta indica* A. Juss., *Datura metel* L. var. *quinquecuspida* Torr., *Ocimum sanctum* L. and *Parthenium hysterophorus* L. was observed *in vitro* study. Leaf extract of *Azadirachta indica* at 100% conc. completely inhibited germination of pathogen spores[117,68]. According to Sharma and Kumar[113], extract of three weed plants, namely, *Capparis decidua*, *Lantana camara* and *Tridax procumbens*, show antifungal property against *Fusarium oxysporum*. Acetone extracts of *Datura stramonium* have been reported to have antifungal activity against several fungi including *Fusarium oxysporum*[41]. In botanical's *Solanum nigrum* L., *Tagetes erecta*, *Polyalthia longifolia*, *Parthenium hysterophorus* L., *Zingiber officinale* was also most effective against *F. udam*[27]. All the plant extracts, viz *Calotropis procera*, *Eucalyptus globulus*, *Jatropha multifida*, *Azadirachta indica*, *Allium sativum* tested, except *Calotropis procera*, were significantly superior in reducing wilt incidence in chickpea[25]. Some local medicinal plants (*Anacyclus valentinus* L., *Artemisia herba* alba Asso., *Eucalyptus* sp, *Inula viscosa* (L.) Aiton, *Laurus nobilis* L., *Mentha pepirita* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Tetraclinis articulata* (Vahl) Masters and *Thymus vulgaris* L.) show antifungal property against F. *oxysporum* f.sp. *lentis*[17]. *Allium sativum* extract, *Azadirachta indica* leaf extract, *Zingiber officinale* Extract, *Calatropis procera* leaf extract, *Moringa oleifera* leaf extract and *Parthenium hysterophorus* L. leaf extract show fungitoxic effect against F. *oxysporum* f.sp. *pisi*[57].

BIOLOGICAL CONTROL METHOD

Most economical and sustainable approach is biological control of this soil borne pathogens. Potential antagonists, especially *Pseudomonas fluorescens* and *Bacillus subtilis* are promising candidates as bio-protectants[95]. The application of a single antagonist is not likely to be better in all environmental conditions where it is applied[79]. Application of *T. viride, T. harzianum, P. fluorescens* and *B. Subtilis* along with neem cake and compost enhanced the survivability of bioagents and suppression of soil borne diseases[80,112]. The most effective species of *Trichoderma* are *T. virens*, *T. viride*, *T. koningii*, *T. polysporum, T. hamatum* and above all *T. harzianum.* These biocontrol agents suppress plant pathogens through a variety of mechanisms especially mycoparasitism, competition, induced resistance, antibiosis etc. Effectiveness of *Trichoderma* strains against a pathogen depends on several factors such as the target fungus, the crop plant and the environmental conditions including soil nutrition, pH, temperature and iron contents[94].

BIOFUMIGATION:

Brassicaceae crop residues have been reported to reduce propagules of soilborne pathogens and result in a concomitant decrease in the incidence of plant diseases caused by them[128]. During the decomposition of crucifer residues, glucosinolates (GSLs) break down to produce sulphides, isothiocyanates (ITCs), thiocyanates and nitrile compounds, which have either fungistatic or fungicidal properties. Soil amendment with crucifer residue combined with solarization produce a greater variety of toxic volatile substances and improve the effectiveness of solarization in reducing pathogen population and thereby disease incidence[103,102].

NANOTECHNOLOGY:

Nanoscale science and nanotechnologies are envisioned to have the potential to revolutionize agriculture and food systems[90] and has given birth to the new era of Agronanotechnology[96]. Nanoparticles have been experimenting as antifungal agents against pathogenic fungi. and have a great potential as 'magic bullets' loaded with herbicides, fungicides, nutrients, fertilizers or nucleic acids and targeting specific plant tissues to release their charge to desired part of plant to achieve the desired results[38]. Different types of nanomaterials like Copper (Cu), Zinc (Zn), Titanium (Ti), Magnesium (Mg), Gold (Au), Alginate and Silver (Ag) have been developed[96]. Against Fusarium oxysporum Ag[61,133], Cu[54,23,127], Zn[98], S[29], ZnO[134] show fungicidal property. According to Duhan et. al.,[38] silver has much higher antifungal activity than that of other metals because block the metabolic process of fungal cell. In future CNT (Carbon nanotube), could be a boon for nanoagrotechnology act as a vehicle to deliver desired molecules into the seeds during germination that can protect them from the diseases. Since it is growth promoting, it will not have any toxic or inhibiting or adverse effect on the plant[96].

Table 1: Morphological Characterization of Fusarium, Fusarium oxysporum and Its formae speciales

S.NO.		MACROMORPHOLOGY	MICROSCOPIC MORPHOLOGY	
			MACROCONIDIA	MICROCONIDIA
1	***Fusarium***	Woolly to cottony white, cream, tan, salmon, cinnamon, yellow, red, violet, pink or purple; on the reverse, it may be colorless, tan, red, dark purple, or brown[110]	long, slender, rather pointed at both end, dorso-ventrally curved, sickle-shaped, septated	appearing as small, usually one-celled spores, oval-shaped, in some species apiculate, tear-drop or pear-shaped and sometimes spherical
2	***Fusarium oxysporum***	floccose, sparse, colour range may be white to pale violet[66]	Short to medium length, straight, slightly curved, slender and thin walled, Usually 3-septate, abundant in sporodochia.	Oval, elliptical or kidney shaped and usually aseptate, Abundant in the aerial mycelia
3	***Fusarium oxysporum*** **f.sp. *ciceri***	floccose, sparse, white[68]	straight to slightly curved, slender and thin walled, 3 or 4 septa.	Ellipsoidal, 0 to 1 septa
4	***Fusarium oxysporum*** **f. sp. *Udum***	Hyaline, selender, branched, White to salmon pink (in mass)[66,93]	Straight to falcate and thin walled, 1-5 septate, but predominantly 3-septate, Curved to almost hooked, Abundant in sporodochia	Fusiform to reniform or oval, white to salman pink, 0 to 1eptate,
5	***Fusarium oxysporum*** **f. sp. *Pisi***	aerial mycelium white, usually becoming purple[9]	fusiform, slightly curved, pointed at the tip, 3-5septate.	abundant, ellipsoidal to cylindrical, straight or curved,
6	***Fusarium oxysporum*** **f. sp. *lentis***	woolly, white, sometimes turning pink[125]	straight to fusiform, falcate, slender, thin-walled, single celled with indistinct septa (rarely 1-5 septate).	Single celled, hyaline, ovoid, cylindrical, oblong or slightly curved

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