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FUSARIUM WILT OF PULSES AND ITS MANAGEMENT BY PLANT PRODUCTS: A REVIEW

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Pulses are annual leguminous crops, which are considered as a major source of protein for the majority of the world population. India is the largest producer as well as consumer of pulses. Chickpea, lentil, pea and pigeonpea are important pulse crops, which are majorly cultivated in India and across the globe. Unfortunately, a range of pathogens has been reported to attack these pulse crops and cause significant damage. *Fusarium* wilt is the most wide-spread disease in legumes growing regions. *Fusarium* Schlechtendahl Emend. Snyder and Hansen are filamentous fungi, belonging to class Ascomycetes and family Hypocreaceae. Among three different asexual spores (viz., microconidia, macroconidia and chlamydospores), chlamydospores serve as primary inoculum in *Fusarium* for the disease occurrence. The pathogen survives in soil, roots, seed and infected plant residues as chlamydospores and mycelium for more than 6 years, which serves as primary inoculum for the development of disease in subsequent seasons. *Fusarium* wilt spread could be limited by using resistant cultivars, soil solarization, disinfection and chemical control methods. However, the management of this soil borne disease still remains difficult. Thus, management of *Fusarium* by employing plant products is an important module of non-chemical based plant disease management. Chemical fungicides may cause serious hazards to plants and the environment. However, plant-based pesticides and metabolites provide sustainable alternatives over chemical fungicides as they are known to have negligible impact on the environment. This review highlights the occurrence and production of four important pulse crops and management of *Fusarium* in these crops by plant products as a promising environment friendly approach.

Keywords: Essential Oil, *Fusarium*, Plant Extract, Pulses, Wilt

Pulses have been cultivated since thousands of years and have been reported from ancient Mesopotamian civilization where peas, beans and lentils were being grown as far back as 8,000 BC. Currently, India is the world's largest producer and consumer of pulses while China, the United States, Brazil, Australia, Canada, Ethiopia, Argentina, Myanmar and Mexico are the major pulse producing countries (Sinha *et al.* 2018). Pulses, popularly known as "Poor man's meat" and "Rich man's vegetable" are major sources of protein, vitamins and important macro and micronutrients (Dwivedi and Sangeeta 2015), thus, serve as an essential component of food security, combating malnutrition, improving human health, alleviating poverty and enhancing agricultural sustainability (Joshi and Rao 2017; Deshmukh and Mogle 2019). However, pulses are vulnerable to several pathogens that cause soil borne diseases such as wilt, collar rot, dry root rot and damping off. *Fusarium* wilt is one of the most prominent diseases of pulses caused by *Fusarium* Schlechtendahl Emend. Snyder and Hansen. It is a filamentous fungus, belonging to Class Ascomycetes and Family Hypocreaceae (Pitt *et al.* 1994). It is a soil borne pathogen, which is common in almost all types of soils causing heavy losses in the crop production. Long term survival of this pathogen in the soil (as chlamydospores) has increased its threat making it a highly devastating disease (Dahal and Shrestha 2018; Rafiq *et al.* 2020). Besides wilt, *Fusarium* causes various other diseases viz., cortical rots, head blights, leaf spots, root rots, fruit rots, cankers, dieback and vascular wilt diseases. However, vascular wilt is the most important disease caused by formae speciales of *Fusarium oxysporum* (Nelson 1964). It affects the water-conducting (xylem) vessels, blocks it completely, so that the plant wilts easily and often die. *Fusarium* wilts are caused by pathogenic strains of several species of *Fusarium*, including *F. eumartii, F. oxysporum, F. avenaceum, F. solani, F. sulphureum* and *F. tabacinum* (Okungbowa and Shittu 2012), which are usually very host-specific. However, the most commonly encountered culprit is *F. oxysporum* (Okungbowa and Shittu 2012). Chemical fungicides are commonly used for the management of wilt disease, but they impart several undesirable effects on soil health and fertility, humans and non-targeted

organisms in the environment. It even leads to the development of chemically resistant strains of the pathogen. Recently, plant-based products are documented as a reliable and safe alternative for the efficient management of plant pathogens. Bioactive compounds from plant origin can be utilized effectively against plant pathogens as they are biodegradable, possess low toxicity and show low risk for pathogen resistance development. This review deals with the production and nutritional importance of major pulse crops, their diseases with a major focus on *Fusarium* wilt and its management by plant extracts and essential oils.

Production and nutritional aspect of important pulse crops : Pulses are grown in all three seasons: i. *Kharif* (July to October): arhar (tur), urd (blackgram), moong (greengram), lobia (cowpea), kulthi (horsegram) and moth; ii. *Rabi* (November to February): gram, lentil, pea, lathyrus and rajmash; iii. *Summer* (March to June) greengram, blackgram and cowpea (Varma 2019). According to the Crop Division, Government of India (GoI, 2018) report, pulses were cultivated over > 29 million ha (Mha) of area and recorded the highest ever production of 25.23 million tonnes (Mt) at a productivity level of 841 kg/ha during 2017-18 (**Table 1**). Ten states viz., Madhya Pradesh (> 8 Mt), Rajasthan (>3 Mt), Maharashtra (>3 Mt), Uttar Pradesh (>2 Mt), Karnataka (2 Mt) and Andhra Pradesh (>1 Mt) followed by Gujarat, Jharkhand, Tamil Nadu and Chhattisgarh are the major producers of pulses, contributing > 90 per cent pulses production. Under the individual crop category, gram recorded the highest ever production of 11.23 Mt at a record productivity level of 1063 kg/ha in an area of 10.56 Mha. Tur (arhar) remained at 2nd position with 4.25 Mt of production in an area of 4.43 Mha at productivity level of 960 kg/ha. Lentil recorded production of 1.61 Mt from an area of 1.55 Mha at productivity level of 1034 kg/ha.

Pulses have a high nutritional value. They are rich in proteins (21g/100 g), carbohydrates (43 g/100 g), fibre, the minerals phosphate, calcium, iron and the vitamins of the B-complex. They are low in sodium and saturated fats. The vegetable proteins in pulses can replace to a great extent animal proteins. This is important for vegetarians who use pulses as a substitute for meat Roy *et al.* 2010.

Common diseases of pulses: Chickpea, lentil, pea and pigeon pea are important pulses attacked by several pathogens causing significant damage to crops. Chickpea is mainly affected by wilt (*F. oxysporum* f.sp. *ciceris* Matuo and Sato), blight (*Mycosphaerella pinodes* B. and Blox), rust (*Uromyces ciceris-arietinii* (Grogn.) Jacz. and Boy.). Lentil is affected by rust (*Uromyces viciae-fabae* (Pers.) J. Schrot), collar rot (*Sclerotium rolfsii* Athelia rolfsii (teleomorph) = *Corticium rolfsii*) and wilt (*F. oxysporum* f.sp. *lentis*). Pea is affected by powdery mildew (*Erysiphe polygoni* DC), wilt (*F. oxysporum* f.sp. *pisi*) and rust (*Uromyces vicia-fabae* (Pers.) Schroet.). Pigeon pea is affected by wilt (*F. oxysporum* f. sp. *udum* (Butler) Snyd. and Hans.) and sterility mosaic. Among these diseases reported, *Fusarium* wilt is widespread across the legumes growing regions (Sinha *et al.* 2018) and is discussed in these four pulse crops.

Chickpea : Chickpea, also known as garbanzo bean or Bengal gram, is an annual diploid (2n = 2x = 16), self-pollinated species (Cobos *et al.* 2007). Only chickpea (*Cicer arietinum* L.) is the cultivated species of 9 annual and about 34 perennial wild species (Singh *et al.* 2008). It has historically been an important daily staple in the diet of millions of people, especially in the developing countries (Diapari *et al.* 2014). The main producers of chickpea are India, Australia and Pakistan, contributing 67.32%, 6.19% and 5.72%, respectively to global production, while countries like Australia, Mexico and Russia are not chickpea consumers but major world exporters (Jendoubi *et al.* 2017). It's the most important pulse crop of

Table 1: FAO report on area harvested, yield and production of pulses in India during year 2018 (FAO 2018)

Pulses	Area harvested (ha)	Yield (hg/ha)	Production (tonnes)
Chickpea	11899185	9564	11380000
Lentil	2215397	7312	1620000
Peas, dry	997735	9226	920473
Peas, green	543000	100000	5430000
Pigeon Pea	5583059	7684	4290000

Table 2: Nutrient content of some important pulses

Pulse	Protein (%)	Carbohydrate (%)	Fat (%)	Calorific value	Calcium (mg/100 g)	Iron (mg/100 g)	P* (mg/100 g)
Chickpea	18-22	61-62	4.5	396	280	12.3	301
Lentil	24-26	57 – 60	1.3	343	69	7	300
Pea	22.5	62.1	1.8	-	64	4.8	-
Pigeonpea	22.3	57.6	1.7	335	73	5.8	304

Source: Tiwari and Shivhare (2016) *: Phosphorus

India and accounts for approximately 75% of world's chickpea production (Biswas and Ali 2017). It is rich source of protein, low in fat and sodium, cholesterol free and an excellent source of both soluble and insoluble fiber, as well as complex carbohydrates, vitamins, folate, and minerals, especially calcium, phosphorous, iron, and magnesium (Roy *et al.* 2010). It consists of an average of 3.0-14.3 mg of iron, 2.2-20 mg of zinc, and 334-446 kcal per 100 g edible portion (Wood and Grusak 2006; Ray *et al.* 2014). These constituents make chickpea a potential staple food to help reduce iron and zinc deficiencies in humans globally (**Table 2**).

Fusarium wilt caused by *F. oxysporum* f. sp *ciceris,* is the most important disease in chickpea reducing its yield (**Table 3**). It is one of the major asexual soil or seed borne disease in chickpea growing under rainfed area (Jendoubi *et al.* 2017). This fungus is pathogenic only on *Cicer* species with high pathogenic variability, with eight races 0, 1A, 1B/C, 2, 3, 4, 5, and 6 (Jiménez-Díaz *et al.* 2015). It is reported to cause severe yield loss in chickpea ranging from 10-100% depending on varietal susceptibility and agro climatic conditions (Warda *et al.* 2017). Wilt symptoms observed after 20-30 days of sowing are termed as "early wilt" causing 77-90% yield decline while symptoms during flowering to the podding stage are known as "late wilt" causing 24-65% decline, respectively (Jiménez-Díaz *et al.* 2015). Kaiser *et al.* (1994) reported 10-40% economic losses in chickpea due to *F. oxysporum* f. sp. *ciceris* worldwide while Khilare *et al*. (2009) reported it to be 10-15%. Dubey *et al.* (2010) reported that disease incidence varied from 14.1-32.0% in the different states of India. The average annual yield losses due to wilt have been estimated to be around 10-90% and sometimes it results in 100% crop loss when the relative humidity is greater than 60% and temperature ranges between 10 and 25°C (Cortes *et al.* 2012).

The isolates of *F. oxysporum* f. sp. *ciceris* from wilted chickpea plants are highly variable in their colony growth pattern, size of colony and

Table 3: Causal organism and symptoms of *Fusarium* wilt of different pulses

Pulse	Botanical name	Common name	Causal organism	Symptoms
Chickpea	*Cicer arietinum* L.	Bengal gram, chana and gram	*Fusarium oxysporum* f. sp. *ciceris*	Seedling gets affected first but in advance stages symptoms of disease may also appear. The plant becomes yellowish and finally dries out. Roots become black and ultimately decompose.
Lentil	*Lens culinaris* Medikus subsp. culinaris	masur, malka (bold seeded)	*Fusarium oxysporum* f. sp. *lentis*	The growth of the plant is checked due to yellowing of leaves, drying of plants. The roots of affected plants remain under-developed and look light brown in colour.
Pea	*Pisum sativum* (L.)	matar	*Fusarium oxysporum* f. sp. *pisi*	Premature yellowing and withering of young leaves during seedling stage and advance stage. Disease causes maximum loss if the crop is sown early.
Pigeonpea	*Cajanus cajan* (L.) Millsp	arhar, red gram, tur	*Fusarium oxysporum* f. sp. *udum*	The leaves on lower branches of the affected plants turn yellow; drop and finally the whole plant dry out. The withering and drying up symptoms appear as if the plants were suffering from drought.

Source: Tiwari and Shivhare (2016)

pigmentations. They also show different pathogenicity and virulence factors. Meki *et al.* (2008) isolated twenty-four isolates of *F. oxysporum* f. sp. *ciceris* from wilted chickpea plants obtained from different districts and 'wilt sick plots' of central Ethiopia to assess variability in pathogenicity of the populations. Based on the reaction types induced on differential lines, isolates were grouped into four corresponding races. Of the 24 isolates, F13, F20 and F22 were the most virulent. Dubey *et al.* (2010) isolated 112 isolates and grouped them into 12 categories on the basis of their radial growth, size of macroconidia and growth pattern. Majority of the isolates were highly pathogenic causing more than 50% wilt in chickpea cultivar JG 62. Alloosh *et al.* (2019) isolated seventy isolates of the wilt pathogen from diseased plant samples collected from farmers' fields and research centers in Syria, and a research station in Lebanon. These isolates were studied for their genetic diversity using random amplification of polymorphic DNA (RAPD), simple sequence repeat (SSR) markers and sequence characterized amplified region (SCAR) molecular markers. Using race-specific markers, four races (0, 1B/C, 5 and 6) were identified and 12 isolates were not designated to any of the known races.

Lentil: Lentil (*Lens culinaris* Medikus) is one of the oldest crops that originated in near East and Mediterranean region and was well known in Egypt and Greece. It has spread to Europe, India and China and now it is introduced and cultivated in most subtropical and warm temperate regions (Kumar *et al.* 2014). Its production is concentrated in the northwest provinces of Australia, Bangladesh, China, Ethiopia, India, Middle East, Nepal, North America, Syria and Western Asia (Bedasa and Zewdie 2019). Lentil is a high value cool season, highly nutritious pulse crop which ranks next to chickpea among rabi pulses having 28.5% mean value of protein (**Table 2**; Stoilova and Pereira 1999). This crop is prone to a number of pathological threats including lentil wilt, stemphilium blight, collar rot and root rot (Yadav 2004). Wilt caused by *F. oxysporum* f. sp. *lentis* is one of the major diseases affecting lentil production across the globe (Pouralibaba *et al*. 2015) resulting yield loss up to 50% in farmer's fields (Tiwari *et al.*

2018). This pathogen can cause infection across all stages of plant growth with higher incidences documented at flowering and podding stage (**Table 3**; Singh *et al.* 1999a; 2015; Chavdarov 2006). *F. oxysporum* f. sp. *lentis* (Fol) isolates exhibit great variability in morphology and aggressiveness. Belabid *et al.* (2004) isolated 32 isolates of Fol from wilted lentil plants from different lentil growing areas in north-west Algeria and performed pathogenicity tests for all isolates. Results indicated that the Fol isolates represent a single race but differ in their aggressiveness on the susceptible lines. Naimuddin and Chaudhary (2009) tested the pathogenicity of 102 isolates of Fol isolated from root samples collected from different areas of Uttar Pradesh and categorized them in 5 groups. Out of 102 isolates, 10 isolates were non pathogenic as they did not induce any mortality in the wilt susceptible plants. Group I had 27 isolates that induced highest wilting (65-80% mortality), whereas group IV and V had only 5 and 9 isolates with 18-30 and 33-38% wilting, respectively. Group II consisted of a maximum number of 32 isolates that caused wilting in the range of 53-63%. Group III had 19 isolates that caused per cent wilting in the range of 42-48. Study reveals that there exists a vast range of variability in pathogenic character of isolates of Fol. Mohammadi *et al.* (2011) isolated 47 isolates from wilted lentil plants collected from different lentil growing areas in Iran and conducted pathogenicity tests. Results showed that the Fol isolates differ in their aggressiveness on the susceptible lines and could be grouped into 3 categories based on bayaa scales. The analysis showed that Fol isolates could be differentiated into 6 groups at 74% similarity, and that could partially separate isolates based on their geographical regions. Most of the *F. oxysporum* isolates tested in the greenhouse had little aggressiveness and included 18 isolates. Sixteen isolates were grouped as highly aggressive while 13 of them were grouped as moderately aggressive. Hiremani and Dubey (2016) isolated seventy five isolates of Fol representing 7 different lentil growing states of India and characterized for their morphological characters and aggressiveness. The Fol isolates were highly variable in their aggressiveness on the susceptible cultivar Sehore 74-3 and caused wilt incidence from 20-80%. Based on wilt incidence, the isolates were categorized into 4 groups. The highly aggressive (>50% wilt) group included the highest number of isolates (29) followed by moderately pathogenic group (>20 to 50% wilt) with 21 isolates. Conversely, Hiremani and Dubey (2018) screened 89 lentil genotypes against virulent isolate of the pathogen (FLS 75) to constitute a set of differential cultivars for race/pathotype determination. The wilt incidence was highly variable ranging from 7 to 93% and 7 genotypes were found resistant, 3 moderately resistant and remaining were susceptible. Considering the reactions, genetic background and area of cultivation, 10 genotypes namely, JL 3, L 4149, PL 4, PL 101, DPL 62, K 75, MC 6, L 6183, Sehore 74-3 and Vidhokar local were selected as differential cultivars for race/pathotype profiling. The virulence of 50 *Fol* isolates was analysed during subsequent years. The isolates belonging to different regions of the country showed marked variability with respect to incidence ranging from 0-100% but the reaction patterns were more or less the same in both the years. Based on the resistant and susceptible reactions on the differential cultivars, the isolates were grouped into eight races/pathotypes and differential cultivar for each race/pathotype was identified.

Pea: Pea (*Pisum sativum* L.) originally cultivated in the Mediterranean basin, is a major annual pulse crop of temperate region. It is the second most important food legume in the world after pigeon pea (Ali *et al.* 2014). Wilt of pea is caused by *F. oxysporum* f.sp. *pisi* (Linford) Snyder and Hansen, and was first recognized during 1918 by Bisby in Minnesota (**Table 3**; Chupp and Sherf 1960). Jones and Linford (1925) first described the disease and also named it "an undescribed wilt disease". At that time, the disease was found in 50 fields in

Wisconsin, and caused severe yield losses in some areas than those reported for root rot. The causal organism was named *F. othoceras* App and Wr var *pisi* in 1928 (Linford 1928) and was later named race 1 of *F. oxysporum* Schl f. sp. *pisi* (van Hall) Snyd and Hans in 1935 (Goth and Webb 1981). Snyder (1932) reported that this pathogen can be transmitted occasionally by seed when harvested from a wilt-infested field. In India it was first reported by Patel *et al*. (1949) from Bombay. Masheshwari *et al* (1982) isolated the pathogen from surface disinfested seeds of 6 varieties grown in the Hoshiarpur district of Punjab, India, where pea root rot and wilt were a major problem. The survey of wilt and root rot complexes in the various pea growing areas in Northern India showed 14-95% loss in Hoshiarpur district of Punjab where disease intensity was 25-100%. There are pathogenic variabilities among foreign isolates of *F. oxysporum* f.sp. *pisi*. Verma and Dohroo (2003) stated that morphological and cultural variability exists among isolates of *F. oxysporum* f.sp. *pisi* collected from pea. These isolates show slow to rapid growth, variable pigmentation and morphology of the hyphae, microconidia, macroconidia and chlamydospores. Four different races of *F. oxysporum* f.sp. *pisi*, viz., 1, 2, 5, and 6, have been described so far in which races 1 and 2 are widely distributed (Infantino *et al*. 2006). Sakoda *et al.* (2019) used three cultivars, *i.e*. Akabana-suzunarisatou (Ak), Misasa (Mi) and Hanaka-kinusaya (Ha), as the sources of Japanese isolates in the study. Five isolates of *F. oxysporum* f.sp. *pisi* (FOP) from three locations of Japan. All five isolates were pathogenic to cultivar Mi.

Pigeonpea

Arhar (*Cajanus cajan* L. Millsp) also known as pigeonpea, red gram is one of the most important leguminous crops and constitutes the chief source of protein for majority of Indian population depending on vegetarian diet (Chaudhary *et al.* 2013). India is the largest producer of pigeonpea (66%) followed by Myanmar (17.09%), Malawi (6.15%), United Republic of Tanzania (5.29%) and Kenya (4.36%). Pigeonpea wilt was first reported from Bihar, India by Butler in 1910 and considered as an important biotic constraint in pigeonpea production in the Indian subcontinent, causing 16-47% crop loss (**Table 3**; Prasad *et al.* 2003). This disease is now widely distributed in India and worldwide (Gupta and Paul 2002).

The disease caused by *F. udum* has been increasing year after year and most of the released cultivars became susceptible indicating the development of more virulent races of the pathogen (Rangaswamy *et al.* 2016). Subramanian (1955) observed considerable variation in cultural characters of *F. udum*. Kumar *et al.* (2017) reported that *F. udum* is widespread in nature and causes heavy yield loss upto 100%. According to Kannaiyan and Nene (1981) loss in individual plants is found to be nearly 100% when wilt occurred at pre-podding stage, 67% at podding stage and 30% at pre-harvest stage. Reddy and Basuchoudhary (1985) demonstrated various isolates of *F. udum* and collected six isolates. They also categorized the isolates into three groups based on radial growth and colony characters. Gaur and Sharma (1989) stated that the eleven single spore isolates of *F. udum* differed in their cultural and morphological characters and also showed a marked diversity in virulence towards the susceptible variety T 21. Okiror (2002) stated that yield loss due to *F. udum* can reach up to 100% loss in grain yield. Kiprop *et al*. (2002) studied in detail about 79 single-spore isolates of *F. udum* collected from Kenya, India and Malawi and characterized them according to their cultural characteristics. The 79 isolates were categorized into two groups of radial mycelial growth and four groups of sporulation. Sinha *et al.* (2008) isolated 69 isolates of *F. udum* from and grouped them in 18 types based on cultural and morphological characters, such as sporulation, mycelial colour, *in vitro* growth rate, length and number of septa of macroconidia. These 69 isolates were also

grouped in 3 types, such as highly, moderately and slow or weakly pathogenic. DNA amplification patterns indicated that an array of highly and moderately pathogenic isolates recovered from various pigeonpea cultivars and sites of northern India produced almost identical patterns. Mishra *et al.* (2014) isolated twenty five isolates of *Fusarium* spp. from major pigeonpea growing areas of Uttar Pradesh. Total isolates were assigned into three groups (on the basis of colony characters, sporulation and degree of pathogenicity test). All the isolates showed strong virulence. Seven isolates (Fu 5, Fu 6, Fu 10, Fu 12, Fu 18, Fu 20, Fu 24) were highly pathogenic (>50% wilt) in which wilting symptoms were noticed 30 days after inoculation.

Fusarium wilt: Historical aspects and its distribution

Fusarium wilt was first reported during the 19th century in pigeonpea by E. J. Butler from India, while its etiology was correctly determined by Padwick in 1940 (Cunnington *et al.* 2007). Based on the structure of the macroconidia with a well distinguished prominent hook this devastating fungus was named *F. udum*. It is host specific to pigeonpea. Butler (1918) and McKerral (1923) indicated the nature of wilt disease to be soil borne and causal organism to be species of *Fusarium.* In 1918 Butler discovered another formae speciales of *Fusarium*, which causes wilt disease in chickpea (*C. arietinum* L.) and named it as *F. oxysporum* f.sp. *ciceri*. Narasimhan (1929) reported the association of *Fusarium* sp. and *Rhizoctonia* sp. with chickpea wilt disease. Later Dastur (1935) found *R. bataticola* as a causative agent of wilt in chickpea and designated it as *Rhizoctonia* wilt. Subsequently, author concluded this wilting was because of physiological reason and assigned it as 'physiological wilt'. Prasad and Padwick (1939) reported *Fusarium* sp. to be the cause of chickpea wilt. The fungus was later named by Padwick (1940) as *F. orthoceras* var. *ciceri.* Erwin (1958) from the U.S.A. reported *F. lateritium* f.sp. *ciceri* to be the causal organism of wilt and questioned the earlier nomenclature as *F. orthoceras* var. *ciceri*. Following the classification of Snyder and Hansen (1940), Chattopadhyay and Sen Gupta (1967) renamed *F. orthoceras* var. *ciceri* as *F. oxysporum* f. sp. *ciceri*. This change has been accepted by Booth (1971). Since then *F. oxysporum* f.sp. *ciceri* is accepted universally as the causal agent of wilt in chickpea. Manucheri and Mesri (1966) observed the association of *F. lateritium* f.sp. *ciceri* with chickpea wilt in Iran. Echandi (1970) from Peru reported that repeated isolation from more than 250 wilted chickpea plants invariably yielded *F. oxysporum* and not *F. lateritium.* Grewal *et al.* (1974) from India for the first time isolated 15.5% *F. oxysporum* f.sp. *ciceri,* 24.7% *F. solani* and 1.6% *F. moniliformae* from wilted chickpea plants. Westerlund *et al.* (1974) reported the association of *F. oxysporum* f. sp. *ciceri* and *F. solani* f. sp. *pisi* in chickpea wilt from California. Cother (1977) observed that *F. orthosporoides* and *F. avennacium* were associated with chickpea wilt from New South Wales. Gupta *et al.* (1986) also reported a number of *Fusarium* spp. associated along with *F. oxysporum* f.sp. *ciceri* in chickpea wilt which caused severe loss of seed production. The *Fusarium* wilt of pea was first recognized during 1918 by Bisby in Minnesota.

In India, wilt diseases alone are empirically estimated to cause about 10 per cent losses in yield, which accounts for approximately 593 thousand tonnes annually (Singh and Dahiya 1973). The disease is widely prevalent throughout India being very destructive in parts of Uttar Pradesh, Madhya Pradesh, Bihar, Punjab and Maharashtra. It is distributed throughout the states wherever the crop is continuously cultivated on the same land for years together. The widespread distribution of *Fusarium* species has been attributed to the ability of these fungi to grow on a wide range of substrates and their efficient mechanisms for spore dispersal (Nelson *et al*. 1994). Its different species are considered to be some of the most important plant disease pathogens,

with some species producing mycotoxins (such as fumonisins, zearalenones and trichothecenes) on plants which contaminate the seeds. From seeds it enters the food chain and affects the human and animal health, and hence are hazardous to agricultural products, wildlife, livestock and humans (Balali and Iranpoor 2006; Arif *et al.* 2011; Wang *et al.* 2011). The genus *Fusarium* currently contains over 20 species (Wang *et al.* 2011). The commonest species include *F. solani, F. oxysporum, F. equisetti* and *F. chlamydosporum* (Burgess *et al.* 1994; Chimbekujwo 2000).

Morphology and disease cycle: Macroscopic and microscopic features, viz., colony colour, length and shape of the macroconidia, number, shape and arrangement of microconidia and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species (Larone 1995; De Hoog *et al*. 2000). Molecular methods, viz., 28S rRNA gene sequencing, may be used for rapid identification of *Fusarium* strains to species and subspecies levels (Hennequin *et al.* 1999). Others are polymerase chain reaction (PCR) based rDNA detection method (Lacmanova *et al.* 2009) and detection of protein banding patterns by SDS-PAGE and esterase isozyme electrophoresis (El-Kazzaz *et al*. 2008).

Three different asexual spores viz., microconidia, macroconidia and chlamydospores are present in *F. oxysporum*. Shape of microconidia is ellipsoidal with 0-1 septa while macroconidia are cylindrical, thin walled, slightly curved with 3-4 septa. The size of microconidia of *F. oxysporum* f. sp. *ciceris* varied from 5.1–12.8 × 2.5–5.0 µm, whereas macroconidia ranged from 16.5–37.9 × 4.0–5.9 µm with 1–5 septations (Dubey *et al.* 2010). The microconidia size of *F. oxysporum* f. sp. *lentis* varies from 5.3-14.8×1.3-5.2 µm whereas macroconidia range between 14.3-46.5×2.5-5.1 µm in size (Hiremani and Dubey 2016). The size of macroconidia of *F. oxysporum* f.sp. *pisi* ranges from 11.6x 3.1 to 25.2 x 6.2 µm and size of microconidia ranged from 3.02x 2.1 µm to 9.2 x 5.6 µm (Kripalini *et al.* 2018). Average size of microconidia of *F. oxysporum* f. sp. *udum* range from 5.33 µm x 2.62 µm and macroconidia range from 22.40 µm x 4.62 µm to 31.83 µm x 3.89 µm (Ghante *et al.* 2018). Chlamydospore is an important spore type and serves as primary inoculum for the disease occurrence (Rafiq *et al.* 2020). Pathogen survives in soil or infected seed between the seasons in the form of free chlamydospores or embedded in plant cells or tissues (Jimenez-Díaz *et al*. 1989; Agrios 2005). These may be found in chains or in pairs also as single cells. It is produced in old mycelia and infects plant residues.

Fungus attacks the root apices or wounded root parts of the plant and penetrates the epidermis and subsequently to the cortex leading to the vascular bundle. Fungus starts to proliferate in the vascular bundle and results in dark brown discoloration. This colonization in the vascular bundle results in the formation of dense gel and causes histological deformation of xylem. The gel and occlusions in vascular tissue plug the normal flow of nutrients and water in the xylem vessel and results in wilting, drooping, yellowing of leaves and ultimately leads to the collapse of whole plant within few days (Jiménez-Díaz *et al.* 2015).

Management of *Fusarium* wilt

Fusarium oxysporum populations are usually controlled through the use of resistant cultivars, cultural practices (as crop rotation, organic matter addition), soil solarization and disinfection, chemical control (Bawa 2016). However, management of this soilborne disease still remains difficult worldwide. Different management methods viz., raised bed preparation; tolerant variety and optimum time of planting prevent the wilt incidence and reduce mortality of wilt (Agrios 2005; Ahmad *et al.* 2012). Sowing date of seed has the greatest effect on incidence of *Fusarium* wilt and yield of pulse crops (Landa *et al.* 2004). Host resistance is the main component of integrated disease management and the development of resistant crop varieties is the

most effective method to manage *Fusarium* wilt. It is the most efficient, cheapest, environmentally safe and economical way of managing *Fusarium* wilt of pulse crops (Bakhsh *et al.* 2007). Efforts have been made at different institutes to locate the source of resistance varieties. The search for sources of resistance to wilt disease in chickpea was first reported by McKerral (1923) who tested 25 chickpea lines against wilt and found 12 cultivars to be resistant against wilt. Since then different cultivars from different places were tested against wilt infection in order to find the resistance varieties (Padwick 1941; Mehta *et al.*1950; Kamboj *et al.* 1990). However, in most of the cases the earlier promising resistance cultivars later became susceptible towards disease (Singh and Dahiya 1973). So far a stable source of resistance against wilt of pulses is not available.

Plant diseases are mostly controlled by chemical pesticides but they are cautioned due to the potential harmful effects on the environment, undesirable effects on non-target organisms and possible carcinogenicity. Therefore, the need for the development of non-chemical alternative methods to control plant diseases is clear (Belabid *et al.* 2010). Considering the adverse effect of chemicals on human health and environment, any alternative of chemical fungicide such as plant products (in form of extract or essential oil) should be practiced and promoted (Sinha *et al.* 2018).

Biological management of plant pathogens by employing plant products has been an important component of non-chemical plant disease management (Kanuri *et al.* 2019). Hence, in recent times application of plant extracts as well as plant metabolites for plant disease management has become an important viable component of Integrated Pest Management. Plant metabolites are eco-friendly where botanicals play an important role (Sahayaraj *et al*. 2009). Plant metabolites and plant based pesticides are considered to be another alternative as they are known to have minimal environmental impact and ill effects on consumers in contrast to synthetic pesticides (Ramaiah *et al.* 2015, Şesan *et al.* 2017). Plant extracts have proved to be complementary control means for soil-borne pathogens (Javaid and Iqbal 2014, Javaid and Rauf 2015).

Plant extracts and essential oils are among the promising strategies not only to manage plant diseases but also to have a safe environment and low toxicity to people due to their natural properties. They have a low risk for resistance development by pathogenic microorganisms. They are also biodegradable compounds and used efficiently in integrated pest management programs. Plant extracts and essential oils have been widely used against bacteria, viruses, fungi and nematodes (Hamad *et al.* 2015).

Plant products to control *Fusarium* wilt of pulses

Plant products viz., extracts and essential oil are substances made by extracting different parts such as leaf, stem, roots etc. of various plants, and often by using a solvent such as ethanol or water. They are used as important biopesticides for management of different diseases and pests of agricultural crops. In this respect various scientists had done important works to evaluate antifungal activity of various plant products against *Fusarium* wilt of pulses. Contributions of some workers are listed below:

Singh *et al.* (1979) evaluated the effect of aqueous garlic-leaf extract on *F. oxysporum* f. sp. *ciceri*, which results in wilt diseases in gram (*C. arietinum*). Results revealed that the growth of the fungi in the liquid medium was greatly reduced by 7,000 and 5,000 ppm of the extract. Germination of gram seeds treated with 50,000-80,000 ppm of the extract was also delayed. Extract-treated seeds sown in soil infested with the pathogens solely and intermixed produced wilt-free seedlings whereas all the seedlings from untreated seeds showed wilt symptoms.

Singh *et al.* (1980) evaluated the effect of aqueous extract and oil of neem (*Azadirachta indica*) on *F. oxysporum* f.sp. *ciceri,* which results in a wilt of gram (*C. arietinum*). Growth of the fungi in liquid medium was inhibited by

extracts of leaf, trunk bark, fruit pulp and oil. Oil-treated seeds sown in soil infested with the pathogens solely and intermixed produced disease-free seedlings whereas all the seedlings from untreated seeds exhibited disease symptoms. Pandey *et al.* (1982) evaluated antifungal activity of *Callistemon lanceolatus* and found that it shows strong inhibitory effect against *F. oxysporum* f. sp. *udum.* Pandey *et al.* (1983) tested 12 essential oils against *F. lateritium* f. sp. *cajani* causing wilt in pigeonpea. The oil of *Ageratum houstonianum* exhibited mycostatic nature at it's MIC of 0.05% but became mycocidal at 0.3%. The oil has broad mycotoxic spectrum, more active than synthetic fungicides and non-phytotoxic to the host plant (*C. cajan*).

Extracts of leaves of 40 plants were evaluated against *F. oxysporum* f. sp. *lentis* causing wilt in lentil, *Mentha spicata* inhibited the mycelial growth of the test pathogen completely. The extract was fungistatic, broad spectrum and remained active up to 6 days at MDAI (1:2 w/v). The extract did not affect the seed germination and morphology of the host plant (Singh *et al.* 1994). Pandey *et al.* (1996) used soil solarization in combination with oilseed organic residues of Neem (*A. indica*) and linseed (*Linum usitatissmum* L.) oilseed meals) to reduce the population of *F. oxysporum* f.sp. *ciceri* and the incidence of chickpea wilt. Solarized plus neem oilseed meal amended soil exhibited maximum reduction in population of the pathogen (99%), and completely eliminated the chickpea wilt. Rana *et al.* (1997) assessed the antifungal activity of essential oil isolated from the leaves of bael (*Aegle marmelos*) using spore germination assay. The oil exhibited 80% fungal growth inhibition at 400 ppm of *F. udum*. Tripathi and Mishra (1997) evaluated 60 plant species against *F. oxysporum* f. sp. *ciceri* causing wilt in chickpea, *Xanthium strumarium* showed absolute toxicity inhibiting mycelial growth of the test fungus completely. The plant has been found to control chickpea wilt up to 88% during soil amendment. Verma and Singh (1997) tested the inhibitory property of *Clerodendrum fragrans* against *F. oxysporum f. sp. udum* and revealed that the leaf extract from *C. fragrans* has antifungal activity. The effect is pronounced only at the lower dilution of 1:100 and the fungi static state is short term, lasting upto 10 days. The possible reason for inactivity of the extract in higher dilutions and short term antifungal state may be that an active substance with antifungal activity may be viable at optimum concentration and is stable under *in vitro* condition. Sharma (1998) tested leaf trunk, leaves and oil extracted from neem tree against *F. oxysporum* f.sp.*ciceri* and found that it contains high inhibitory action against test pathogen. Rai and Acharya (1999) evaluated 31 plants belonging to Asteraceae family against the chickpea wilt pathogen *F. oxysporum* f. sp. *ciceris* and found that these plant extracts were very effective in inhibiting the growth of fungi. Singh *et al.* (1999b) evaluated the effect of aqueous, methanol, petroleum ether, chloroform and ethyl acetate extracts of rhizomes of a tropical weed *Cyperus rotundus* on the spore germination of *F. udum*. Ethyl acetate extract exhibited inhibitory effect on spore germination at 1000 /-lg mr'. Fractionation of ethyl acetate extract on silica gel columns resulted in eluents rich in flavonoids and terpenes.

Singh and Tripathi (2000) surveyed different districts of Eastern Uttar Pradesh for wilted samples of lentils and isolated 15 fungi out of which *F. oxysporum* f. sp. *lentis* was dominant. Leaf extract of *Artabotrys hexapetalous* showed 100% activity against test pathogen. The 10 and 15% leaf extract amended soil controlled the wilt of lentil caused by *F. oxysporum* f. sp. *lentis* up to 82 and 98%, respectively. Shukla *et al.* (2002) had done *in vitro* screening of some essential oils against *F. oxysporum* Schlecht and *F. udum* Butler which cause wilt diseases of pigeonpea (*C. cajan* (L.) Millsp.). Results revealed that the oil of *Trachyspermum ammi* (ajowain) was a strong fungitoxicant. The toxicity of the oil was found to be fungicidal at 0.1%, which inhibited heavy doses of inocula (25 fungal discs, each of 5-mm diameter) and killed the test pathogen in just 2-3s. The oil did not have any phytotoxic effects

on seed germination and seedling growth of the host plant, *C. cajan*. The oil's toxicity prevents the wilt disease of pigeonpea at 10% concentration and also has been reported to be harmless to human health. Singh and Chand (2004) conducted an experiment to determine the efficacy of extracts from *Calotropis procera*, *Datura stramonium*, *Eucalyptus globulens*, *Jatropha multifida*, *A. indica* and *Allium sativum* on the spore germination of *F. oxysporum* f.sp. *ciceri*. They found that the leaf extract of *A. indica* (100%) completely controlled spore germination of fungus. Farshbaf *et al.* (2004) studied the composition and antifungal effects of *Mentha piperita* oil on *F. oxysporum* f. sp. *ciceri* and found that 1600 ppm treatment completely inhibited the growth of fungus after 3 days of study. Essential oil analysis with GC/MS revealed that main compounds of oil include menthol (19.76%), menthan-3-one (19.31%), menthofuran+isomenthone (9.12%), 1, 8-cineole+beta phellandren (8.8%) and menthol acetate (5.63%).

Pourbaig *et al.* (2004) investigated the antifungal effect of essential oil thyme (*Thymus vulgaris*) on *F. oxysporum* f.sp. *ciceri*. The minimum inhibitory concentration (MIC) of the extract was observed at 400 ppm. Concentrations of 800 and 1600 ppm results in 100% inhibition of fungal growth. The major compounds of thyme oil were found to be Terpinen (4.65%), Cymene (12.16%), Thymol (19.8%), Linallol (4%) and Caryophyllene (4.07%). According to Chand and Singh (2005) various plant extracts viz., *C. procera*, *E. globulus*, *J.multifida*, *A. indica*, *A. sativum* shows significant effect in reducing wilt incidence in chickpea.

Rao and Tripathi (2005) screened 40 plant species against *F. oxysporum* f. sp. *pisi* causing wilt in *P. sativum*. *Piper methysticum* showed absolute toxicity and was fungicidal at 1:30 w/v dilution with no adverse effect on the host plant. During *in vivo* studies Piper leaves controlled wilt up to 90% in plastic pots, however, in field conditions the extract controlled the disease upto 80%. Mukhtar (2007) evaluated the antifungal effect of aqueous extracts of 4 plant species viz; *A. indica* A. Juss., *Datura metel* L. var. quinquecuspida Torr., *Ocimum sanctum* L. and *Parthenium hysterophorus* L. and stated that all 4 plant extracts at 40% concentration were effective in controlling the mycelial growth of *F. oxysporum f. sp. ciceri*. Mandhare and Suryawanshi (2008) tested the efficacy of some botanicals against *F. udum* in the laboratory. The results indicate that extracts of *O. sanctum, Eucalyptus* spp. and *Nerium indicum* completely inhibited the growth of fungus on agar as well as potato dextrose broth. The growth of *F. udum* was inhibited by 80, 75 and 71 per cent with extract of *A. indica, L. usitattisimum* and *Hibiscus rosasinensis* respectively. The extract of *Vitex negundo, Sorghum bicolor, Piper nigrum, Vinca rosea* and *Zea mays* were also effective and inhibited the growth of *F. udum* by 60, 60, 60, 63 and 62 per cent, respectively. In another experiment, Mandhare and Suryawanshi (2008) reported that extract of *A. sativum* (15%) inhibited the growth of *F. oxysporum* f. sp. *ciceri* completely while the extract of *A. indica* inhibited the growth of the same fungus by 55.5%.

Sahani and Saxena (2009) stated that the antifungal activity of ethanolic extracts of bark of *Euphorbia nerifolia* shows absolute toxicity against *F. oxysporum* f sp. *pisi.* and resulted in complete inhibition on the growth of mycelium. According to Sharma and Kumar (2009), extract of three weed plants, viz., *Capparis decidua, Lantana camara* and *Tridax procumbens*, showed antifungal property against *F. oxysporum.* They further concluded that free flavonoids of *L. camara* (flower), *C. decidua* (fruit), *T. procumbens* (stem, leaf), bound flavonoids of *T. procumbens* (leaf, flower) and alkaloids of *T. procumbens* (flower) showed excellent inhibitory potential against the test pathogen. Belabid *et al.* (2010) evaluated the antifungal activity of powders and essential oils formulation of some local medicinal plants (viz., *Anacyclus valentinus*, *Artemisia herba-alba*, *Eucalyptus* sp, *Inula viscosa*, *Laurus nobilis*, *M. piperita*,

Rosmarinus officinalis, *Salvia officinalis*, *Tetraclinis articulata* and *T. vulgaris* on *F. oxysporum* f. sp. *lentis* population in the soil. Results obtained showed that the treatments 10 and 5% with the powders of *I. viscosa* and *M. piperita* and the essential oils formulation in all treatments have significantly reduced the soil population densities of Fol and the disease incidence on lentils. They also observed reaction in the pathogen population and increase of healthy plants, which indicates that these extracts could have an important role in biological based management strategies to control *Fusarium* wilt disease in lentils.

Mehta *et al.* (2010) reported that garlic bulb (*A. sativum*) extract at 5% concentration possesses a high inhibition effect on the growth of *F. udum.* Singh *et al.* (2010) reported that *A. indica* has the highest inhibition on radial growth of *F. udum* (68%). Behtoei *et al.* (2012) evaluated antifungal activity of three essential oils viz., *Bunium persicum* (Parsi Zira), *Carum copticum* (Ajwain) and *Cinnamomum zeylanicum* (cinnamon) and found that essential oil of *C. zeylanicum* at 200 ppm concentrations completely inhibited the growth of *F. oxysporum* f. sp. *ciceri*. Shukla and Dwivedi (2012) evaluated *in vitro* efficacy of different plant extracts viz. bitter gourd, turmeric, garlic and black pepper to control different *Fusarium* species viz. *F. udum* (causing wilt in pigeonpea) and *F. oxysporum* f.sp. *ciceri* (causing wilt in chickpea). All the plant extracts recorded considerable reduction in the growth of test pathogens. Growth of *F. udum* has been reduced by 15% concentration of turmeric (89%) followed by garlic (88%) and black pepper (82%). In case of *F. oxysporum* f.sp. *ciceri*, 15% concentration of garlic, turmeric and black pepper reduced the growth upto 95%, 88% and 78%, respectively.

In another study done by Devi and Chhetry (2012), *A. sativum* was found to be the most effective extract in controlling *Fusarium. A. sativum* at 20% alone recorded 100% inhibition of mycelial growth. Minz *et al.* (2012) tested antifungal potential of aqueous extracts of forty plants of different families against *F. oxysporum* f.sp. *ciceri* causal agent of wilting of chick pea. Among forty plant species tested aqueous extract of *Chenopodium ambrosioides* showed significant antifungal activity against the test fungus. Ali *et al.* (2013) evaluated the plant extracts (viz. *Aloe vera, L. camera, Eclipta alba, Mentha arvensis, O. sanctum, A. indica, Datura fastuosa, Tegetes erecta* and *P. hysterophorus*) and essential oils (viz., *M. pipreta, Nicotiana* spp. *Dalbergia sissoo, Cedrus deodara, Myristica fragrans, Cassia tamalo, Foeniculum vulgare, Eucalyptus* spp. *Trachhyspermum amni, A. indica, Psoralea corylifolia, Elettaria cardiamomum* and *Olea europaea*) against *F. oxysporum* f. sp. *pisi.* Extract of *A. vera* was most effective in inhibiting mycelial growth (69%) of *F. oxysporum* f. sp. *pisi* followed by *L. camera* (50%), *E. alba* (49%), *M. arvensis* (31%), *O. sanctum* (26%) and *A. indica* (20%). The inhibitory effect of the leaf extracts against *F. oxysporum* f. sp. *pisi* might be attributed due to the presence of antifungal compounds viz. Azadirachtin in *A. indica*, Eucalyptol in *E. citridora*. Among different essential oil tested *M. piperita, Nicotiana* spp., *D. sissoo, C. deodara, M. fragrans, C. tamala, F. vulgare* and *Eucalyptus* sp. completely inhibited the growth of *F. oxysporum* f.sp. *pisi* and these were followed by *T. amni* (80%) and *A. indica* (78%).

Chaudhary *et al.* (2013) evaluated the relative efficacies of ten plant extracts (viz., makoy, marigold, ashoka, parthenium, ginger, bakayan, aak, dhatura, garlic, tulsi) belonging to different families against *F. udam* causing wilt of pigeonpea under laboratory conditions and found that the makoy possesses highest inhibitory effect (76%) while tulsi have lowest (4%) inhibitory effect on fungal growth. They also tested seven different neem products viz., achook, nemark, neem gold, econim, neem kernel, javan and nemata on fungal growth of *F. udam* and found that achook (5%) has good inhibitory effect on fungal growth and results in 85% reduction in growth rate when tested under *in vitro* condition. The antifungal activity of some plant extracts (viz., garlic,

ginger, eucalyptus, coriander, onion, oak) *in vitro* and *in vivo* condition against lentil wilt caused by *F. oxysporum* Schechet. f. sp. *lentis* have been tested by Garkoti et al. (2013). All the botanicals tested were found effective on test pathogen. However, garlic extract seed treatment followed by ginger, showed lowest disease incidence, highest grain yield as well as maximum 1000-grains weight in comparison to check plot. Hossain *et al.* (2013) had done an *in vitro* test to determine the effect of aqueous extract of three cost-effective and commonly available botanicals such as *Zingiber officinale* rhizome, *Allium cepa* bulbs and freshly harvested *A. indica* leaves on colony growth of *F. oxysporum* f. sp. *ciceris* and found that *A. indica* leaf extract recorded maximum inhibition (55%) of radial growth of *F. oxysporum* f. sp. *ciceris* at all concentrations tested. The bioactivity of *A. indica* extracts are attributed by various compounds like nimbin, nimbidin and salannin, while the most important antifungal compound is azadirachtin (Lale and Abdulrahman 1999). The *A. indica* leaf extract may also produce volatile and nonvolatile substances during their decomposition in the soil and cause both volatile and nonvolatile fungistatic effects against soil borne pathogenic fungi (Dubey *et al.* 2009). Khaleel *et al.* (2014) evaluated the fungitoxic effects of six methanolic plant extracts viz; garlic extract, ginger extract, neem leaf extract, moringa leaf extract and parthenium leaf extract against mycelial growth of *F. oxysporum* f.sp. *pisi* causing wilt disease in pea. Neem leaf extract proved to be the best antifungal agent, as it had given the maximum colony reduction (61%) followed by ginger extract (58%), while parthenium leaf extract proved to be the least effective (25%) at highest concentration (1000 µg/ml).

Kumar *et al.* (2014) tested 6 different essential oils viz., citronella oil, geranium oil, jatropha oil, menthol oil, mustard oil, neem oil against *F. oxysporum* f.sp. *lentis* with different concentration (2.5, 5.0 and 10.0%) and calculated the percent inhibition of spore germination after 24, 48 and 72 h at 25±1°C temperature and observed that seed treatment with menthol oil (50ml/kg seed) had significant response with respect to disease incidence (0.56% and 1.32%), 1000-grain weight (13.73 g and 13.67 g) and grain yield (603.10 and 503.50) kg/h followed by geranium oil (50 ml/kg seed) which showed disease incidence (0.82% and 1.51%),1000 grain weight (13.38 g and 13.03g), grain yield (584.50 and 475.20) Kg/ha, citronella oil (50ml/kg seed) resulted in disease incidence (1.03% and 2.15%),1000 grain weight (12.53 g and 12.50 g), grain yield (517.30 and 465.00) kg/ha while jatropha oil (50ml/kg seed) showed high disease incidence(1.42% and 3.42%), 1000 grain weight (11.80g and12.03g), lowest grain yield. Dwivedi and Sangeeta (2015) analyzed the antifungal potentiality of aqueous extract of five medicinal plants viz., *Tinospora cordifolia*, *Cymbopogon citratus*, *Moringa oleifera*, *Z. officinale* and *T. ammi* against *F. oxysporum* f. sp. *ciceri* causing chickpea wilt. All the five aqueous plant extracts significantly inhibited the growth of the test pathogen. The percent inhibition range was between 77-92 after eight day of study. At 25% concentration, the highest percent inhibition was recorded by *C. citrates* (leaves) (84%) followed by *Z. officinale* (rhizome) (79%), *T. cordifolia* (leaves) (78%), *M. oleifera* (bark) (74%) and *T. ammi* (seeds) (57%), respectively. Gawai (2015) tested antifungal activity of essential oil of *C. citratus* (lemon grass) against *F. oxysporum* f. sp. *udum* and found that lemon grass oil resulted in complete inhibition of fungal growth. From the result it could be concluded that the essential oil from the leaves of *C. citratus* may be rich in antifungal compounds, which possess considerable antifungal properties and a number of biological and medicinal potentials. Maghraoui *et al.* (2015) reported that essential oil of *A. herba-alba* possesses high fungicidal property toward *F. oxysporum* f. sp. *ciceris* (Chickpea *Fusarium* Wilt). They further

concluded that chemical constituents of *A. herba-alba* such as Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)- (30%), 1,3-Cyclopentadiene, 1,2,5,5 tetramethyl- (16%), Eucalyptol (7%) in the oil, which imparted antimicrobial property. Srivastava and Dwivedi (2015) evaluated antifungal activity of aqueous and alcoholic extracts of *C. lanceolatus, Tamarindus indicus, Terminalia arjuna* and *Zizyphus jujube* against *F. oxysporum* f.sp. *ciceri.* Alcoholic extracts of all the tested plants were superior as compared to their water counterparts across the concentrations used. Alcoholic extract of *C. lanceolatus* was found to be the most effective at 500 ppm, among the tested plant varieties inhibiting the growth to (98%) followed by *Z. jujube* (71%), *T. indicus* (62%) and *T. arjuna* (59%), respectively.

The antifungal effects of the medicinal plant extracts argel (*Solenostemma argel*), ginger (*Z. officinale*) and jatropha (*Jatropha crucus*) (seeds, stems, leaves and roots) were determined *in vitro* by Mohamed *et al.* (2016) using aqueous and ethanolic extracts following the poisoned food technique. All extracts gave significant inhibition of growth of *F. oxysporum* f. sp. *ciceris*. Among all plants tested, argel extract was the most effective followed in descending order by ginger and jatropha. Furthermore, the ethanolic extracts of all plants were more suppressive to the fungal growth than their aqueous equivalents. Gupta *et al.* (2017) studied 15 plant extracts viz., *A. sativum, A. indica, Achyranthus aspera, Adhatoda vasica, Citrus limon, Cassia occidentalis, Euphorbia hirta, Ricinus communis, A. cepa, Curcuma domestica, C. zedoaria, Oxalis curniculata, Cannabis sativa, D. stramonium* and *Vernonia cinerea* for their antifungal potential against wilt causing fungus *F. oxysporum* f. sp. *udum*. The result revealed that out of 15 plants the extract of *Curcuma zedoaria* recorded maximum inhibition up to 100%, followed by *A. indica* (81%) and *A. cepa* (68%). Herode *et al.* (2017) tested citronella oil (*Cymbopogon winterianus*) against *F. oxysporum* f. sp. *ciceri* and found that it possesses antifungal activity at different concentrations 400, 800, 1000, 1200, 1400 ppm per ml. The higher concentration at 1400 ppm per ml recorded the zone of inhibition at 11.74 mm. Jalander and Mamatha (2017) evaluated antifungal activity of aqueous and ethanolic leaf extracts of 6 different medicinal plants viz., *A. vasica, A. indica, Catharanthus roseus, C. citratus, E. globules* and *O. sanctum* against *F. oxysporum* f.sp. *udum* in pigeonpea. The results indicated that the ethanolic leaf extracts showed better inhibitory activity than aqueous leaf extracts against plant pathogenic fungus tested. The ethanolic leaf extract prepared from *A. indica* recorded better efficacy against wilt pathogenic fungus. In order to evaluate the efficacy of various plant extracts against the *Fusarium* wilt of pigeonpea caused by *F. udum,* Kumar *et al.* (2017) tested 8 plant extracts viz., neem, mehandi, tulsi, beal, marigold, bulb of garlic, onion and ginger. The highest percent disease control was observed to be 94% to 97% in garlic while lowest 49% to 58% in marigold. Garlic (67%), garlic + tulsi (58%) and tulsi (50%) gave more than 50% disease control at 120 days after sowing.

Patra *et al.* (2017) tested various oil seed cakes viz., sesame cake, mustard cake, neem cake, groundnut cake, vermi-compost and spent mushroom substrate (sms) to find out their effect on wilt disease in chickpea caused by *F. oxysporum* f.sp. *ciceri*. All the treatments showed positive results towards disease management. Among the seven treatments of soil amendments, neem cake was found to be most effective in terms of disease incidence (11%) and was followed by vermi-compost, groundnut cake, sesame cake, spent mushroom substrate (SMS) and farm yard manure (FYM) with 12.06 %, 12.94%, 13.76%, 14.43 % and 15.12 % incidence respectively. The least effective treatment was mustard cake with 16.07 % disease incidence. The neem cake gave the highest yield (1665 kg/ha) followed by vermi-compost 1637 kg/ha. Farah *et al.* (2018) investigated the antifungal activity of three essential oils viz., *M. spicata, Cymbopogon schoenanthus* and *Citrus*

reticulata by disc diffusion method against *F. oxysporum* f. sp. *ciceri* (causing wilt in chickpea) and all concentrations showed complete inhibition of tested fungus.

Teia and Osman (2018) studied 6 different plants viz., *A. sativum*, *A. indica, Capsicum frutescence, Momordica balsamina, Petroselinum crispum* and *Pulicaria undulata* against *F. oxysporum* f.sp. *ciceri*, the causal agent of vascular wilt in chickpea. Among these only *A. sativum* and *P. crispum* showed high antifungal activity while other plants showed moderate activity.

Chaudhary *et al.* (2019) tested 7 plant extracts against *F. udum* causing pigeonpea wilt and found that all tested extracts possessed significant antifungal activity. The most effective extract was *A. sativum* showing 62.8% inhibitory effect, followed by *A. indica* (34.4%), *Polyalthia longifolia* (27.6%), *P. hysterophorus* (26.4%), *R. communis* (17.3%), *C. limon* (13.5%), *A. cepa* (12.2%).

Moutassem *et al.* (2019) evaluated six aromatic plants species viz., thyme (*Thymus pallescens*), wormwood (*A. herba-alba*), laurel (*Laurus nobilis*), pine (*Pinus halepensis*), lemongrass (*C. citratus* and the Peruvian pepper tree (*Schinus molle*) against *F. oxysporum* f.sp. *ciceris*. As for the mycelium growth and sporulation, essential oil of *T. pallescens* and *C. citratus* showed the highest inhibition (100%) of the spore germination of the *F. oxysporum* f.sp. *ciceris.* Essential oil of *L. nobilis* and *S. molle* also exhibited a potent inhibitory effect on the spore germination of the *F. oxysporum* f.sp. *ciceris* (36.02–100% and 32.39–83%, respectively). *P. halepensis* and *A. herba-alba* presented the lowest level of inhibition regarding the *F. oxysporum* f.sp. *ciceris* spore germination. According to the results, the major constituents of the essential oil of *T. pallescens*, *C. citratus, S. molle, L. nobilis, A. herba-alba* and *P. halepensis* were carvacrol (54.09%), geraniol (21.86%), α-phellandrene (16.27%), 1,8-cineole (40.77%), camphor (24.6%), β-caryophyllene (28.43%), respectively which impart their fungicidal property.

Mode of action of plant products: Studies on the antifungal mechanism have been primarily focused on the following two actions: (i) the formation of transmembrane pores or ion channels on the cellular membrane leading to the leakage of essential metabolites, and (ii) the disruption of the cell wall structure, interfering with cell wall synthesis (Hu *et al.* 2010). Ergosterol is an important component of membrane lipids that modulates the fluidity, permeability and thickness of the membrane. These sterols have important roles in membrane organization and function and preferentially associate with sphingolipids in microdomains (Maxfield and Tabas 2005, Munro 2003). Absence of ergosterol in fungal cells results in abnormal functioning of fungal cell membrane and even leads to cell rupture. Ergosterol combines with phospholipid to stabilize the membrane structure, which can regulate the mobility of the fungal cell membrane and plays an important role in ensuring the integrity of the membrane structure, membrane-bound enzyme activity, cell viability and cellular transport (Georgopapadakov and Walsh 1994). Plant based antifungal products inhibit the synthesis of ergosterol in fungal cells (Ghannoum and Rice 1999, Bentz and Six 2009). According to Mostafa *et al*. (2018), the difference in effect of plant extracts is due to variation in their chemical constituents and volatile nature of their components.

a. Mode of action of plant extracts: The cell walls of fungi are very unique and are critical for its survival. It is important to identify the inhibitors of fungal cell wall synthesis and assembly based on osmotic support and morphological character of the cell. Any cell wall active agents lyse the cell by damaging cell wall components in fungi (Khan and Nasreen 2010). Treatment of mycelia with the crude extract of plants shows plasmolysis, distortion and squashing of cells. Hyphae appear empty, collapsing and completely dead while the untreated mycelia shows well-

developed inflated cells, normal cell wall and normal conidia (Hashem *et al.* 2016). Plant extracts disrupt the synthesis of critical proteins and enzymes, which may ultimately inhibit the growth of fungi. It interrupts the fungal cell membrane systems and inhibition of ergosterol synthesis and the respiratory chain (Chen *et al.* 2018).

b. Mode of action of essential oils: Essential oils are hydrophobic in nature. They can enter in the phospholipid bilayer of the cell wall and mitochondria distorting the structure and making them more susceptible to cell leakage (Badawy and Abdelgaleil 2014). Essential oils are typically a mixture of different terpenoid compounds and their oxygenated derivatives (Wijesekara *et al.* 1997). These oils are known for their broad spectrum antifungal activity against both human and plant pathogens (Daferera *et al.* 2003; Isman 2000; Pandey *et al.* 2003). The antifungal essential oils reduce hyphal growth and induce lysis and cytoplasmic evacuation in fungi (Fiori *et al.* 2000). Growth inhibition by essential oils often involves induction of changes in cell wall composition (Ghfir *et al.* 1997), plasma membrane disruption, mitochondrial structure disorganization, and interference with enzymatic reactions of the mitochondrial membrane, such as respiratory electron transport, proton transport, and coupled phosphorylation steps (Knobloch *et al.* 1989).

CONCLUSION

Now a days increasing use of various types of chemicals to control plant pathogens/diseases is very common. Synthetic fungicides efficiently remove the phytopathogenic fungi and result in better crop yields. They have immediate effects on pathogens and help in its complete removal but parallely impart lots of ill effects on plant and soil health. Moreover, development of fungicide tolerant strains of pathogens and accumulation in the food chain resulting in the process of biomagnification of these chemicals are major concerns associated with synthetic fungicides. The widespread use of chemicals in agriculture is a serious matter of public concern. Besides its disease suppression ability, it is equally important to analyze its potential harmful effects on the environment and undesirable effects on non-target organisms. With rising concerns on the adverse effect of synthetic chemicals on the environment as well as on human health, a reliable alternative to these chemical fungicides is warranted. There are various methods other than using synthetic chemicals that can be used to control plant diseases. However, plant based products are considered as the most effective and reliable. Plant based products cause no adverse effect on the environment and are safe for the consumers. Medicinal and aromatic plants are an important source of natural bioactive compounds having microbicidal properties. The antimicrobial activities of plants are majorly due to their chemical composition and the nature of major volatile compounds present. Use of plant extracts and essential oils has emerged as the most promising strategies to control plant diseases. Conversely, natural plant extracts have very low risk for resistance development by pathogenic microorganisms and are also biodegradable, which makes them suitable for the integrated pest management programs. Agricultural production of pulses can be increased significantly across the globe by a better management of major pathogenic diseases such as *Fusarium* wilt. Using plant extracts for this purpose is indeed a promising alternative and in line with sustainable approach to enhance crop production without any side effect on ecosystem and consumers.

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