WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 5.210

Volume 4, Issue 09, 1242-1249 Research Article ISSN 2278 – 4357

DETECTION OF SEED MYCOFLORA FROM CHICKPEA WILT COMPLEX SEEDBORNE *FUSARIUM OXYSPORUM* F.SP. *CICERI* DISEASED SEEDS

***Lily Trivedi and Y. P. S Rathi**

Department of Plant Pathology, G.B. P.U. A & T, Pantnagar. Pin 263145.

*Jayoti Vidyapeeth Women's University, Jaipur.

Article Received on
14 July 2015,

Revised on 04 Aug 2015,
Accepted on 26 Aug 2015,

***Correspondence for Author**
Lily Trivedi
Jayoti Vidyapeeth Women's University, Jaipur.

ABSTRACT

Chickpea wilt complex is associated with numerous pathogens, among which *Fusarium oxysporum* f.sp. *ciceri* is the major pathogen. The fungal species isolated in Agar Plate were belonging to 6 genera namely, *Aspergillus niger*, *Penicillium* , *Fusarium moniliforme* (was absent in Pant G114*)*, *Fusarium oxysporum* f.sp. *ciceri*, *Rhizopus sp*. and *Trichoderma harzanium* (were absent from genotype ICCL 87322 and variety H208) whereas fungal species isolated in the blotter test were i.e. *Aspergillus niger, Penicillium* , *Fusarium moniliforme* (was absent in Pant G114*)*, *Fusarium oxysporum* f.sp. *ciceri*, *Rhizopus sp*. and *Trichoderma harzanium.* However, *Trichoderma harzanium* was absent in case of variety H208 and genotype ICCL 87322. Since seedborne infections attack in the initial stage of seed germination therefore, disease effect was observed on seedling emergence, seedling mortality, radical length and plumule length. Four varieties/genotypes of chickpea:- Local Variety , Pant G-114 variety, ICCL 87322 genotype and H 208 variety isolated *Fusarium oxysporum* f.sp. *ciceri* as internal seed borne infection . On artificial inoculation, maximum loss to all treatments was in H208 whereas minimum in Pant G 114.

KEYWORDS: *Cicer arietinum* L. ,Chickpea wilt ,Seed mycoflora, *Fusarium oxysporum* f.sp. *ciceri.*

INTRODUCTION

Chickpea (*Cicer arietinum* L*)* is India's major winter pulse crop. It is third most important grain legume in the world . Among the soil borne disease of chickpea, 'Wilt Complex' is of great significance in 'Terai' area of Uttar Pradesh as it inflicts several yield losses. Several fungal pathogens are associated, such as *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Macrophomina phaseolina* but *Fusarium oxysporum* f.sp. *ciceri* is the most serious one.[8,9] The seedborne diseases deteriorate the quality of chickpea seeds, causing discoloration and spoilage of seeds. These seeds further infect the healthy seeds and spread the pathogen in disease free area and seedborne diseases causes changes in the seed quality , also a number of fungi *Alternaria alternate, Aspergillus flavus, A.niger, Curvularia lunata, F.moniliforme, Helminthosporium sativum, Mucor sp., Penicillium notatum* and *Rhizopus nigrans* associated. [1,8,13,14] The legume seed borne fungi was obtained by using blotter plate method from selected untreated and treated seeds. [11] The untreated seeds were found to be associated with highest number of seed borne fungi. The fungi isolated from these seeds are *Alternaria alternata, Chaetomium spp., Penicillium citrinum, Aspergillus niger, A. fumigatus, A. flavus, Rhizopus nigricans, Fusarium oxysporum, F. moniliform , F. solani, Chaetomium* sp, *Curvularia lunata, Macrophomin sp, Monilia* sp., *Penicillium* sp., *Rhizoctonia sp, Trichoderma etc*.[11] Other workers also used standard blotter paper, agar plate and seed washates methods. Among the three methods, the agar paper method was found to be suitable as in less incubation; there was higher percent incidence of seed mycoflora.[10] The present investigations were undertaken to find out the seed mycoflora associated both externally and internally seedborne, from four genotypes/varieties seeds taken from wilt infected chickpea plants.

MATERIAL AND METHODS

Seeds of four genotypes were used for the study namely –Local variety, H208, ICCL87322 and Pant G114. All the experiments were conducted *in vitro* as well as *in vivo.* The seedborne infection of various fungi was determined by different methods as described by ISTA.[7] with modifications. Observation of dry seed was conducted under stereo binocular microscope for any fruiting body/spore and variation in the shape, colour and appearance of the seed due to infection. Further, washing test were conducted for the presence of any spores, oospores and teliospores or any other fruiting structure by using sterilized water and shaken over a mechanical shaker for 10min .The liquid was taken and centrifuged at 3,000 rpm for 20 min. The sediment was further then examined under compound microscope.

Blotter Method & Agar Method were used for seed borne mycoflora. For internally seed-borne infection, seeds were surface sterilized by immersing in mercuric chloride solution (0.1%) for 2 min and subsequently rinsed 3 times in sterilized distilled water. Both unsterilized and surface sterilized seeds were separately placed on Blotter in Petri plates at the rate of 10 seeds/plate with an equal distance. Agar plates with P.D.A as medium was used. Both unsterilized and surface sterilized seeds were separately placed on solidified P.D.A surface at the rate of 10 seeds/plate with an equal distance. These Petri plates were incubated in B.O.D incubator at 25 $\pm 2^{o}$C for 8 days under 12hrs alternating cycles of near ultra-violet (NUV) light and darkness. Thus, the seeds exposed were examined for fungal growth on the 3rd, 7th and 9th day of incubation at 25 $\pm 2^{o}$C. Data was taken on 100 seeds per variety. Sterile conditions were maintained by using Laminar flow and sterilized water.

Purification and Identification- Different fungi obtained from chickpea seeds after 7 days were isolated and purified .The pure culture ,thus obtained identified primarily on the basis of colony characteristics, spores, shapes, size and pigmentation and finally confirmed through microscopic examinations. The identified fungi were then tested for pathogenicity by inoculating them on healthy chickpea seeds. *Fusarium oxysporum* f.sp. *ciceri* isolated was inoculated on chickpea seeds.

For seed germination Towel paper method was used. Twenty-five seeds were placed at an equal distance over the towel paper which was subsequently covered by another moistened towel paper size. Thereafter, the towel papers along with the butterpaper were rolled up. One hundred artificially inoculated seeds were used for each treatment (4 replications). They were then kept, upright at 25 $\pm 2^{o}$C. in incubator for 7 days and then observed the effect on seed germination, seedling mortality, radical and plumule length. The inoculated seeds were also sown in glasshouse for confirmation of symptoms in plants. The soil was autoclaved at 121^{0}C for 2 hrs and then filled in 22cm diameter plastic pots. Five seeds in each pot were sown. Twenty days after sowing the typical wilt symptoms of wilt were seen and thus, observations were recorded. Uninoculated healthy seeds served as control.

Statistical Analysis used is 2 factorial C.R.D, except in Table No. 3.

Table 1: Seed borne fungi isolated through Blotter Method.

S.no	Fungi isolated	Varieties/genotypes							
		Local Variety		Pant G-114		ICCL 87322		H 208	
		US	SS	US	SS	US	SS	US	SS
1.	*Aspergillus niger*	+	-	+	-	+	-	+	-
2.	*Penicillium*	+	-	+	-	+	-	+	-
3.	*Fusarium moniliforme*	+	-	-	-	+	-	+	-
4.	*Fusarium oxysporum* f.sp. *ciceri*	+	+	+	+	+	+	+	+
5.	*Rhizopus sp*.	+	-	+	-	+	-	+	-
6.	*Trichoderma harzanium*	+	-	+	-	-	-	+	-

US-UNSTERILIZED, SS- SURFACESTERILIZED

Table 2: Seed borne fungi isolated through Agar-plate Method

S.no	Fungi isolated	Varieties/genotypes							
		Local Variety		Pant G-114		ICCL 87322		H 208	
		US	SS	US	SS	US	SS	US	SS
1.	*Aspergillus niger*	+	-	+	-	+	-	+	-
2.	*Penicillium*	+	-	+	-	+	-	+	-
3.	*Fusarium moniliforme*	+	-	-	-	+	-	+	-
4.	*Fusarium oxysporum* f.sp. *ciceri*	+	+	+	+	+	+	+	+
5.	*Rhizopus sp*.	+	-	+	-	+	-	+	-
6.	*Trichoderma harzanium*	+	-	+	-	-	-	-	-

US-UNSTERILIZED, SS- SURFACESTERILIZED

Table 3. Per cent seed infection of *Fusarium oxysporum* f.sp. *ciceri* present as internal seed borneinfection in diseased varieties/genotypes of chickpea

S.no.	Varieties/genotypes	Per cent seed infection
1.	Local	9.25 %
2.	Pant G 114	3.00 %
3.	ICCL 87322	1.25 %
4.	H 208	4.20 %

4. Effect of *Fusarium oxysporum* f.sp. *ciceri* on artificially inoculated seeds for per cent seed germination and percent seedling mortality, radical and plumule length (cm)

S.no.	Varieties/genotypes Characters	Treatments	Local	Pant G 114	ICCL 87322	H 208
1	Percent seed germination	INOCULATED SEEDS	42.61	51.38	51.38	41.50
		UNINOCULATEDSEEDS	55.95	71.65	60.74	49.04
2	Percent seedling mortality	INOCULATED SEEDS	37.81	18.85	19.22	38.04
		UNINOCULATEDSEEDS	22.75	15.21	12.76	25.82
3	Radicle length (cm)	INOCULATED SEEDS	0.425	0.450	0.450	0.475
		UNINOCULATEDSEEDS	0.675	0.500	0.700	0.575
4	Plumule Length (cm)	INOCULATED SEEDS	0.475	0.475	5.33	5.45
		UNINOCULATED SEEDS	3.50	4.50	3.33	3.75

CD for inoculated seeds varieties at 5 % = 3.49, CD for uninoculated seeds varieties at 5 %=0.09

CD for inoculated seeds interaction at 5 %= 4.94, CD for uninoculated seeds interaction at 5 %=0.81

CD for inoculated seeds treatments at 5 %= 7.33, CD for uninoculated seeds treatments at 5 %=0.06

RESULTS AND DISCUSSION

Diseased seeds were smaller, wrinkled and discolored as healthy seeds compared to which were larger in size, smooth and light coloured.[6] The washing test, revealed spores of *Fusarium oxysporum*, *Fusarium moniliforme* and *Aspergillus sp*. The Blotter Method showed the presence of the following fungi- *Aspergillus niger*, *Penicillium* , *Fusarium moniliforme* (was absent in Pant G114*), Fusarium oxysporum* f.sp. *ciceri* , *Rhizopus sp*. and *Trichoderma harzanium* (absent from genotype ICCL 87322 and variety H208,Table 1). Other workers have also found these fungi along with other fungi like *Alternaria* sp., *Curvularia* sp., *Helminthosporium*, etc in their experiments .[8,11,13,14] In surface sterilized seeds, only *Fusarium oxysporum* f.sp. *ciceri* was present in all the varieties**.** The Agar plate method results were similar in all varieties, except in variety H208 and genotype ICCL 87322. where *Trichoderma harzanium* was absent.Previous workers have also reported the presence of these fungi.[3,11,13,14] However in surface sterilized seeds, only *Fusarium oxysporum* f.sp. *ciceri* was present in seeds of all the chickpea varieties included in the present study, thus it indicates it's internal seed borne nature. On the diseased sample seeds, *Fusarium oxysporum* f.sp. *ciceri* per cent seed infection was recorded from seeds in all four varieties of chickpea. The per cent seed infection was minimum (1.25%) in genotype ICCL87322 and maximum (9.25%) in the local variety. The plants infected with *Fusarium*

oxysporum f.sp. *ciceri* contain less genera of fungi while healthy plants contain other fungi also like *Penicillium* and *Trichoderma* sp.[2] The variation in number and fungal species associated with different samples could be largely attributed to the differences in agro-climatic variation ,soil types, germination multiplication, perpetuation and dispersal of fungal spores.[4] The colony characteristics of the isolated fungi appeared as white cottony growth which became felted and wrinkled in old culture colonies. Microconidia were oval to cylindrical, straight to curved and measured 2.5-3.5 x 5-11µm.

Macroconidia with lesser number than microconidia borne on branched conidiophores, were thin walled, 3-5 septate, fusoid and pointed at both ends, and 3.5-4.5 x 25-65 µm. Chlamydospores were smooth walled, both terminal and intercalary, solitary in pairs or in chains confirming it to be *Fusarium oxysporum* f.sp. *ciceri.*

S**eed germination** reduced significantly in artifically inoculated seeds with *Fusarium oxysporum* f.sp. *ciceri* as compared to uninoculated seeds. The minimum seed germination was observed in variety H 208 & local variety. The maximum seed germination was in variety Pant G114. **Seedling mortality** was maximum was in variety H 208 and local variety. The minimum seedling mortality was in variety Pant G114 closely followed by genotype ICCL87322. Variety H208 & the local variety proved to better. Similar, trend of results could be due to different response of different varieties due to their susceptible and resistant reactions and thus, indicating more seed borne infection in susceptible variety.[14]

The **radical length** was reduced in all inoculated seeds of all the varieties tested as compared to uninoculated seeds. However, the result related to **plumule length** were *vice-versa* of radical length. In artifically inoculated seeds of each variety the plumule length was more as compared to untreated seeds. However, the difference in plumule length among each variety was insignificant. This may be because the pathogenic fungi *Fusarium oxysporum* f.sp. *ciceri* produces metabolites which cause extensive seed rot and abnormal seedling growth in chickpea.[5] These seeds when grown showed typical wilt symptoms in mature plants causing the affected plants lose to their turgidity and droop down. Drooping started from upper portions of plants. Leaves turned yellow and started drying .When stems of the wilted seedling were split open from collar region downwards, black discolouration of internal tissue was seen as described in wilt disease.[8,12]

CONCLUSION

The present investigations revealed a number of fungal species isolated from seeds of wilt complex diseased seeds. *Fusarium oxysporum* f.sp. *ciceri* in surface sterilized seeds confirmed its internal seed borne nature . Due to the effect of fungus , radicle length of seeds reduced, whereas plumule length increased in artifically inoculated seeds as compared to uninoculated seeds. Among all the varieties , Pant G114 and genotype ICCL 87322. proved to best.

REFERENCES

1. Chattopadhyay, Nabanita., S.A.K.M. Kaiser and Prasant K. Sen Gupta. Effect of soil factors on competitive saprophytic activity and crop sequence on the population of three soil borne fungal pathogens of chickpea. *Ann.Pl. Protec.Sci*., 2001; 9: 237-243.
2. Dhedhi, B.M.;Om Gupta and V.A. Patel. Association of micro-organisms in rhizosphere and rhizoplane of healthy and wilted chickpea plants. *Indian J. Mycol. Pl. Pathol*., 1990; 20: 72-73.
3. Dwivedi S.N and T.N Shukla. Mycoflora of gram seeds in different agro-climatic conditions and their pathology. *Indian Phytopathol*., 1990; 43: 98-99.
4. Dwivedi, S.N and T.N Shukla. Effect of seed treatment on seed mycoflora, germination, plant stand and vigour of gram (*Cicer arietinum* L.). *Pesticides*., 1989; 23: 30-32.
5. Gupta, O and N.D. Sharma. Effect of common soil fungal metabolites on chickpea seed germination and root length. *International Chickpea and Pigeonpes Newsl.*, 1995; 2: 28-29.
6. Haware, M.P.; Nene, Y.L. and S.B. Mathur. Seedborne diseases of chickpea. Technical Bulletin No.1 from The Danish Institute of Seed Pathology for Developing Countries. Copenhagen, Denmark in collaboration with ICRESAT: 1986; 32.
7. ISTA.International rules for seed testing, seed science and technology, 1976; 4: 3-49.
8. Jmenez R.M.-Diaz ;Pablo Castillo,Maria del Mar Jmenez-Gasco,Blanc B.Landa and Juan A. navas-Casta. Fusarial wilt of chickpea : Biology, ecology and management. *Crop Protec.*, 2015; 73: 16-27.
9. Kaur, N.P and A.N. Mukhopadhyay. Integrated control of chickpea wilt complex by *Trichoderma* and chemicals methods in India. *Tropical Pest Management*, 1992; 38 : 372-375.

10. L. R. Rathod, M. D. Jadhav, S. K. Mane, S. M. Muley, P. S. Deshmukh. Seed Borne Mycoflora Of Legume Seeds. *International Journal of Advanced Biotechnology and Research.*, 2012; 3(1): 5.
11. Narayan M. Ghangaokarl and Ayodhya D. Kshirsagar. Study Of Seed Borne Fungi Of Different Legumes. *Trends in Life Sciences,*2013; 2(1): 32-35.
12. Nene,Y.L.; Shiela,V.K. and S.B.Sharma. A world list of chickpea (*Cicer arietinum* L.) and Pigeonpea (*Cajanus cajan* (L.) Mill sp.) pathogens. ICRISAT,Hyderabad,India, Pulse Pathology Progress Rep.,1985; 32: 1-19.
13. Riyaz Ahmad Khan; Towseef Ahmad Bhat and Krishan Kumar . *Asian J Pharm Clin Res, Vol 6, Suppl*., 2013; 1: 211-212.
14. Singh, Kiran,; Singh.A.K.and R.P.Singh. Detection of Seed Mycoflora of Chickpea(*Cicer arietinum* L.). *Ann. Pl. Protec.Sci*., 2005; 13: 167-171.