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RESEARCH ARTICLE

STUDIES ON SEED TRANSMISSION AND MANAGEMENT OF *FUSARIUM OXYSPORUM* F.SP. *CICERI* CAUSING CHICKPEA WILT

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ABSTRACT

Chickpea (*Cicer arietinum* L.) an important pulse crop of India. The major diseases of the chickpea crop are Ascochyta blight, *Fusarium* wilt and dry root rot. Of these wilt caused by *Fusarium oxysporum* f. sp. *ciceri* has been considered as divesting one to cause up to 10 per cent loss in yield. Symptoms of the disease in the field and in pathogenicity test were described. The pathogen was isolated and its pathogenic ability was established as per Kochs postulates morphological & cultural characters have been described and discussed. The pathogen has adversely affected the seed germination and seedlings emergence and caused wilting in 34 plants out of 54 seedlings emerged during pathogenicity test. The pathogen was detected in the all-susceptible varieties of chickpea ranging from 12-20 present with maximum in ICP-1454 followed by Radhey and ICC-1876. It was not present in resistant variety except Avarodhi. Varieties ICC-203 and ICCV-32 did not carry seed infection. Pre-treatment of the seeds with the sodium hypochlorite and present in the seed coat of all infected seeds, followed by cotyledons and embryonal axis, pathogen survived in the seed of variety Radhey for 11 month, in ICP-1454 and ICC-1876 for 8 month. The pathogen was transmitted from seed to seedlings within 15 days in the ratio 9:1 to 4.5:1 with in variety Radhey. The pathogen was found systemic as it was recovered from the healthy tissues as well diseased tissues from roots to the top of the seedlings in serial isolation. Companion and Bavistin T were completely inhibited the fungal growth of the pathogen in vitro. Other fungicides viz., Bavistin, Vitavax, Thiram, Mancozeb, Cuman L, and Capton were such effective in checking the fungal growth in vitro.

Key words: Chickpea, *Fusarium oxysporum* f. sp. *Ciceri*, Pathogenicity, Fungicide

INTRODUCTION

Pulse crops play an important role in Indian agriculture, besides being rich in protein, they sustain the productivity of the cropping system. Their ability to use atmospheric nitrogen through biological nitrogen fixation (BNF) is economically more sound and environmentally acceptable. Pulses are grown 60 per cent in *Rabi* and 40 per cent in K*harif*. In India the total food grain production during 2016-2017 was 272 million tones. Out of this pulses contributed 22.22 million tone). India is the largest producer, 25% of world's production, and consumer 27% of total pulses of the world. The availability of pulses per capita is 47 gm./day. Gram (Chana) (*Cicer aritinum* L.) belongs to the sub family Papilionaceae of the family Leguminaceae is a herbaceous annual, having branching close to the ground with semi-erect to semi-spreading habit. Its centre of origin is considered to be in the tract lying between Cancasus and Himalayas from where it has spread to different countries including India. Sprouted seeds are recommended for breakfast. Its green leaves produces malice and oxalic acids, which are prescribed for intestinal disorders. Gram possesses a highly economic nutritive value as is evident from its analysis.

One hundred-gram chickpea seeds contain about 9.8 per cent moisture, 21.1 g protein, and 5.3 g fat. 61.0 g carbohydrates and 3.9 g fiber in addition to vitamins and other things. Taking into consideration the rapid growth of population and alarming situation of protein deficiency in the country, there is need of coordinated efforts by the breeders, agronomists and plant pathologists for making improvement of this important pulse crop. Apart from other reasons, the main cause of low yield of this crop is the incidence of diseases. However, there is ample scope for increasing average production of this crop and every effort has to be made for it by adopting plant protection measures and package of practices. This crop (chickpea) is subjected to attack by a number of fungal, viral, bacterial and nematode diseases. Out of these maladies, wilt alone causes considerable loss to gram in India every year. It is a complex problem and various pathogens as well as physiological factors are implicated to cause wilt of gram, which lead to variation in disease syndrome, although often there is overlapping of symptoms. However, the typical vascular wilt caused by *Fusarium oxysporum f. sp. ciceri* is the outstanding problem in Uttar Pradesh. So far, inadequate information is available on the *Fusarium* wilt of gram as regards to seed-borne nature and management of the disease through agronomical practices in India.

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MATERIAL AND METHODS

Symptomatology

Detailed disease symptoms starting from the disease appearance to crop maturity were observed. Critical observation of the symptoms on roots, collar, bark, internal tissues and foliage etc., were recorded both under natural and artificial inoculated condition. Diseased gram plants showing characteristic wilt symptoms were collected from Legume Research Farm, C.S. Azad University of Agriculture & Technology, Kanpur in the last week of November, 2002 and March, 2003 and brought to the laboratory for the critical examination.

Isolation, purification, identification and pathogenicity test of the causal organism.

A. Isolation and purification of the pathogen: For isolation of the pathogen wilt affected gram roots were used. The roots were first washed in tap water to remove dust particles. The roots were cut into small pieces with a sterilized razor and sterilized in 0.1 per cent mercuric chloride solution for one minute. Thorough washing was done in sterilized water for three times to remove the mercuric chloride. Later these root pieces were transferred in between two folds of sterilized blotter papers to remove the excess of water. These dried root pieces were finally transferred on 2 per cent Potato Dextrose Agar (PDA) medium in Petri plates with the help of a sterilized pointed inoculating needle or forceps in the inoculation chamber. These Petri plates were incubated at 28-30 ^{0}C for the growth of the pathogen. As soon as the mycelial growth was visible the growing mycelium in different Petri plates was transferred and purified by repeated transfer of growing hyphal tips to the sterilized culture tubes and Petri plates containing 2 per cent PDA medium.

B. Identification of the pathogen: The fungus was grown on Potato dextrose agar medium. The measurements of different morphological structures were done under the microscope. Observations on the following morphological characters of the fungus were noted.

(i) Mycelial characteristics: Colour, branching pattern and width of the hyphae etc., will be studied under microscope for identification.
(ii) Sporodochia: Its presence, colour, shape, size and arrangement were recorded under the compound microscope.
(iii) Microconidia: Number, branching pattern, size, shape etc., will be observed under microscope for specific identification.
(iv) Macroconidia: These will also studied under microscope for number, colour, septation, size and arrangement for specific identification.
(v) Chlamydospores: Studies on the position, arrangement, shape, and morphology of the chlamydospores will be done for better understanding about the pathogen.

C. Pathogenicity Test: Pathogenicity test of the isolates obtained from affected gram roots was done on the same host to establish the pathogenic nature of the fungus. The experiment was carried out in 10 inches pots filled with ordinary loam soil which was sterilized for four hours in autoclave at 1.1 kg/cm2 pressure before inoculation. The inoculums were prepared by growing the pure culture of the fungus on sand corn meal medium for 3 weeks. Inoculation of soil was done seven days before sowing of the seeds by thorough mixing of soil with fungus culture in pots. The culture was added @ 5 per cent of the weight of the soil in the pots and the soil in pots without adding culture medium served as control. Healthy seeds of the host were firstly disinfected with 0.1 per cent mercuric chloride solution for two minutes and then rinsed with sterilized water, dried and sown in the pots. The pots were watered as and when required to maintain the moisture. The pathogenicity test was done by another method also. In this case, the sterilized soil was filled in pots. When the seedling emerged and attained the height of about 10 cm, the agar disc (1.5 cm diameter), containing the young mycelial culture of the pathogen on potato dextrose agar (PDA) medium was placed near base of the plant and pressed down to about 0.5 cm. below the surface. The pots having 10 seeds each were kept under each treatment. Presence of disease and germination were recorded 15 days after sowing. The plants were observed daily for development of symptoms after emergence. Final observations were recorded two months after sowing by removing the plants from soil and gently washed the roots in tap water. The pathogen was reisolated from the internal tissues of infected plants on potato dextrose agar medium for confirming of Koch's Postulate (Koch, 1882).

Assessment of *Fusarium oxysporum* f. sp. *ciceri* in seeds of different verities of chickpea

The study of seed infection and the pattern and growth habit of *Fusarium oxysporum* f. sp. *ciceri* colony was critically observed on seed *in situ* by using Standard blotter method (ISTA, 1999). These observations were also recorded by Agar plate method (Neergaard, 1979) for the confirmation of results obtained by blotter method. For both methods (SBM and APM) six varieties (3 susceptible and 3 resistant) namely ICP-1454, ICC-1876, Radhey (susceptible), ICC-203, Avarodhi, and ICCV-32 (resistant to disease under field condition) were taken.

A. Standard blotter method: Three hundred seeds were used for each variety. Four replication each of 50 seeds were taken for each sample. The seeds of each variety were randomly selected from properly homogenized sample and were divided in to three lots, each of hundred seeds. The homogenization of each sample was done by mixing and dividing the seeds in precision divider (Gampet type). First lot of one hundred seeds remained in treated; second was pretreated with 1% sodium hypo chloride solution for 10 minutes and third one was pretreated with 0.1% mercuric chloride solution for 1 minute. Four, replications were used for each variety, each replication consisted 25 seeds. Ten randomly selected seeds of each treatment were placed at equal distance with the help of sterilized forceps on three layers of moist blotter papers in each 90 mm Polypropylene Petri plates and incubated at 25 ± 10C under alternating cycle of 12 hours NUV (Near Ultra violet) light and 12 hour darkness for seven days. On eighth day the seed, were examined under stereo binocular microscope (6.4-40x) for the presence of *Fusarium oxysporum* f. sp. *ciceri*. The number of seeds bearing the colonies of *Fusarium oxysporum* f. sp. *ciceri* were counted and recorded and interpreted as per cent infection of the *Fusarium oxysporum* f. sp. *ciceri*.

B. Agar plate method: In this method potato dextrose agar (PDA) medium was used in place of blotters. PDA was

prepared and sterilized at 1.1 kg/cm2. Fifteen ml. of medium was poured in each Petri-plate and allowed to solidify. Four drops of lactic acid was added in each flask containing 200 ml of PDA before pouring in Petri-plates in order to check the bacterial contamination. One hundred fifty seeds were taken from each sample (variety) and randomly divided in to three lots: Untreated, pretreated with 1% NaOCl for 10 minute and pretreated with 0.1% HgCl2 for 1 minute each of 50 seeds. Five randomly selected seeds (Untreated and Pretreated) were placed at equal distance with the help of sterilized forceps on 90 mm PDA Petri-plates. Over all replications were maintained for each treatment. The plates were then incubated at 25 ± 10C under the alternating cycle of 12 hours NUV (Near Ultra Violet) light and 12 hours darkness for seven days. On eighth day, the growth of the fungus on seeds was examined macroscopically by naked eyes and then under stereo binocular and compound microscopes for specific identification of the pathogen. The number of seeds bearing the colonies of *Fusarium oxysporum* f.sp. *ciceri* were counted, recorded and interpreted in per cent infection of the *Fusarium oxysporum* f. sp. *ciceri*.

Location of the pathogen in different seed components of chickpea

Seeds of chickpea varieties like ICP-1454, Radhey, and ICC-1876 collected from post padding wilted plants and used for studying in the location of *Fusarium oxysporum* f. sp. *ciceri* in seed components on potato dextrose agar (PDA) medium by using component platting method (Maden *et al.*, 1975). One hundred seeds of each variety were soaked individually in tube containing sterilized distilled water for 24 hours. The seeds were later dissected individually in to different components *viz.*, seed coat, cotyledons and embryonal axis with the help of sterilized needle and forceps under aseptic condition. These seed components were divided into two categories surface sterilized and surface unsterilized with aqueous solution of sodium hypo chloride (1 per cent available chlorine) for 10 minutes. These seed components were tested by agar plate method for the presence of *Fusarium oxysporum* f. sp. *ciceri*. The components showing growth of the pathogen were recorded and interpreted in term of per cent infection in seed components of the infected seeds.

Study of survival of pathogen in seed

Since the pathogen is seed borne. Its survival in the seed was studied. The seeds were collected from wilted plant at maturity of susceptible variety ICP-1454, Radhey and ICC-1876 stored in the paper bags under ambient conditions (25 ± 1^0C) from May, 2003 to April 2004. Monthly isolations were taken from the seeds by agar plate method to know the viability of the pathogen in seed.

Study the transmission of the pathogen from seed to seedling

A. Seedling symptoms test: The transmission of the pathogen from seed to seedling was studied by seedling symptom test (Khare et al., 1977) followed by serial isolation method (Neergaard, 1979) on variety ICP-1454, Radhey, and ICC-1876 under laboratory conditions. In seedling symptom test one hundred seeds of each variety were surface sterilized with 1 per cent sodium hypo chlorite solution for 10 minutes and placed aseptically in separate culture tubes containing 2 per cent water agar medium (One seed in one tubes). These tubes were plugged loosely with cotton and placed in an incubation room at 25 ± 1 ^{0}C under 12 hours alternating cycle of artificial fluorescent light 12 hours darkness for 45 days. Observations on the symptoms on seed and seedling were recorded in the tube after 7 days and up to 45 days and calculated in terms of the ratio of seed infection of seed transmission. Isolations from the diseased tissues and rotted seeds were taken serially on PDA in serial isolation test for confirming the association of the pathogen and the root to apical tip of the stem and placed on potato dextrose agar medium in Petri- plates and incubated for 7 days at 25 ± 1 ^{0}C in serial isolation method.

Management of the disease by chemical methods

Six fungicides viz., Bavistin 50 WP, Thiram 75 DS, Vitavax, Cuman L, Mancozeb, Captan, and two combinations of fungicides *viz.*, Companion (Carbendazim 12% + Mancozeb 63%) and Bavistin T (Carbendazim + Thiram in ratio of 1:2) were assessed for their efficacy against *F. oxysporum* f. sp. *ciceri* through laboratory bio-assay, using poison food technique (Schmitz, 1930). Requisite quantity of the fungicides was incorporated in 2 per cent Potato Dextrose Agar (PDA) medium, which was shaken well to make it homogenous. The medium was then poured into 90 mm Petri-plates. The experiment was conducted in completely randomized design with three replications. Suitable control was also maintained by having plate with addition of no fungicides. A circular disc of 5 mm diameter taken from 15-day old culture of the pathogen was cut by sterilized cork borer and placed in the center of each Petri-plate containing solidified fungi toxicant mixed medium and control (check) without fungi toxicant. After inoculation the Petri/dishes were incubated at 25 ± 1^0C for 15 days. Final observations on radial growth were taken when Petri-plates of control were fully covered with the growth of fungus. The efficacy of fungi toxicants was assessed by measuring the growth of the colony diameter in mm and interpreted in per cent inhibition over control by following formula.

$$\text{PI (per cent inhibition)} = \frac{\text{Growth (mm) in control (-) Growth (mm) in treated plates}}{\text{Growth in control}} \times 100$$

RESULTS AND DISCUSSION

Chickpea *(Cicer aritinum* L.) is an important pulse crop of India. It is cultivated on 6.10 million hectares with production of 5.27 million tones and productivity of 8.65 q/ha. Its productivity is quite low due to several biotic and abiotic stresses. Among the biotic stress, diseases are the major constraints. The major diseases of the chickpea crop are Ascochyta blight, *Fusarium* wilt, dry root rot. Of these wilt caused by *Fusarium oxysporum* f. sp. *ciceri* has been considered as devastating one to cause up to 10 per cent loss in yield every year. The disease can be characterized by the presence of patches of dead seedlings or adult plants in field. Initial symptoms of the disease in the field were drooping of plants followed by sudden death. Transverse section of the basal system of the roots when observed under the microscope fungal mass and discoloration of vascular cells were observed in the vascular bundles. It was noticed that the disease assumed serious proportions at Kanpur, only when the plants were about one month old, particularly in case of early sown crop

and again during the times of flowering in plants during February and March when temperature started rising. There by indicating that high temperature plays important role in the development of disease. The findings have close concurrence the findings of Nene *et al.* (1979). The pathogen was isolated from the disease materials obtained from wilt-sick plot of the Legume Research Form, C.S.A. University of Agriculture and Technology, Kanpur during November 2002 and March 2003 on 2 per cent potato dextrose agar (PDA) medium and later purified on Potato dextrose agar medium. The colonies of the fungus on Potato dextrose agar medium were white, radiating with wine-red pigmentation in the later stage which was clearly visible from the bottom side of the Petri plate. The mycelium of the fungus was profusely branched, creeping, hyaline, cylindrical, septate and measuring 3.20 to 4.6 m in width. The fungus produces white to light orange sporodochia on potato dextrose agar medium which was rarely produced but if produced it was completely covered by arial mycelium. The microconidia of the fungus were hyaline, single celled, oval to cylindrical, straight to slightly curve and measuring 2.5-3.5 x 5-11µm in size. The macroconidia of the fungus were produced on branched conidiophores. They were fucoids with pointed ends, hyaline, septate (3-4 septate) and measuring 3.0 - 4.5 x 20 - 55 µm. The Chlamydospores were produced in the old culture, which were globose to sub globose, thick walled smooth surfaced and measuring 7.0 - 8.0 x 3.5-5.0µm. Fungus were found similar the morphological characters of the presents to that reported by Booth (1971).

The pathogen has adverse effect on the seed germination and seedling emergence. The seed germination was reduced by 11.85 %. Out of 75 germinated seeds 54 seedlings developed and 34 plants showed wilting during pathogenicity test. The symptoms of wilting started appearing after 15 days of sowing in the seedlings as drying of seedling from the tip and 20% seedlings got infected within two months where as the wilting was recorded at the time of adult stage in 14 plants only. The pathogen was reisolated from the disease tissues of the roots of chickpea, on and comparing with the original isolate was found similar to original isolate of the pathogen thus proving Koch's postulate (1882). Out of six seed samples, collated from susceptible & resistant cultivars tested for the presence of the pathogen by using standard blotter & agar plate methods, the pathogen was detected in the all susceptible varieties of chickpea ranging from 12-20 per cent. Standard blotter methods had an edge over agar plate method so far as the detection of the pathogen was concerned. The pretreatment of the seeds with the sodium hypochlorite and mercuric chloride reduced the recovery of the pathogen from the infected seeds. The average recovery percentage from sodium hypochlorite and mercuric chloride pre-treated seeds were 12.17 and 4.67 per cent, respectively. The seed infection was maximum in the susceptible variety ICP-1454 (20 %) and minimum resistant variety Avarodhi (3%) whereas two resistant varieties ICC-203 and ICCV-32 did not carry the pathogen. The above finding is similar with the findings of Haware *et al.* (1978), Khune and Patil (1992) and Gangwar (2004). Component plating method of infected seeds of variety Radhey revealed that the pathogen was present in all the components of infected seeds. It was present in the seed coat of all the infected seeds and then entered into the deep tissues of cotyledons and embryonal axis to the extent of 65.04 and 45.01 per cent, respectively. The infection in the seed components varied within the varieties. It was maximum in ICP-1454 and minimum in ICC-1876. The foregoing experiment indicated that the pathogen was carried with the chickpea seeds externally on its surface in the form of mycelium fragments and micro and micro conidia and internally in the tissue of seed coat, cotyledons and embryonal axis. The ratio between seed coat: cotyledons: embryonal axis was 2.22: 1.44: 1. These results are in the accordance with the studies of Haware *et al.* (1996) and Gangwar (2004), who reported that the pathogen is systemic in nature and present all the seed components. The pathogen survives in the seeds of three chickpea varieties viz., ICP-1454. Radhey and ICC-1876 from 8 to 11 months. There was prolong survival of the pathogen recorded in the variety Radhey for 11 months, while in ICP-1454 and ICC-1876 for 8 months. The results indicated that the pathogen was able to survive in the seeds during off-season. The finding has closed concurrence with the findings of Haware *et al.* (1978), Gangwar (2004) reported that the pathogen survived in the form of Chlamydospores in the helium region of seed for 9 to 13 months.

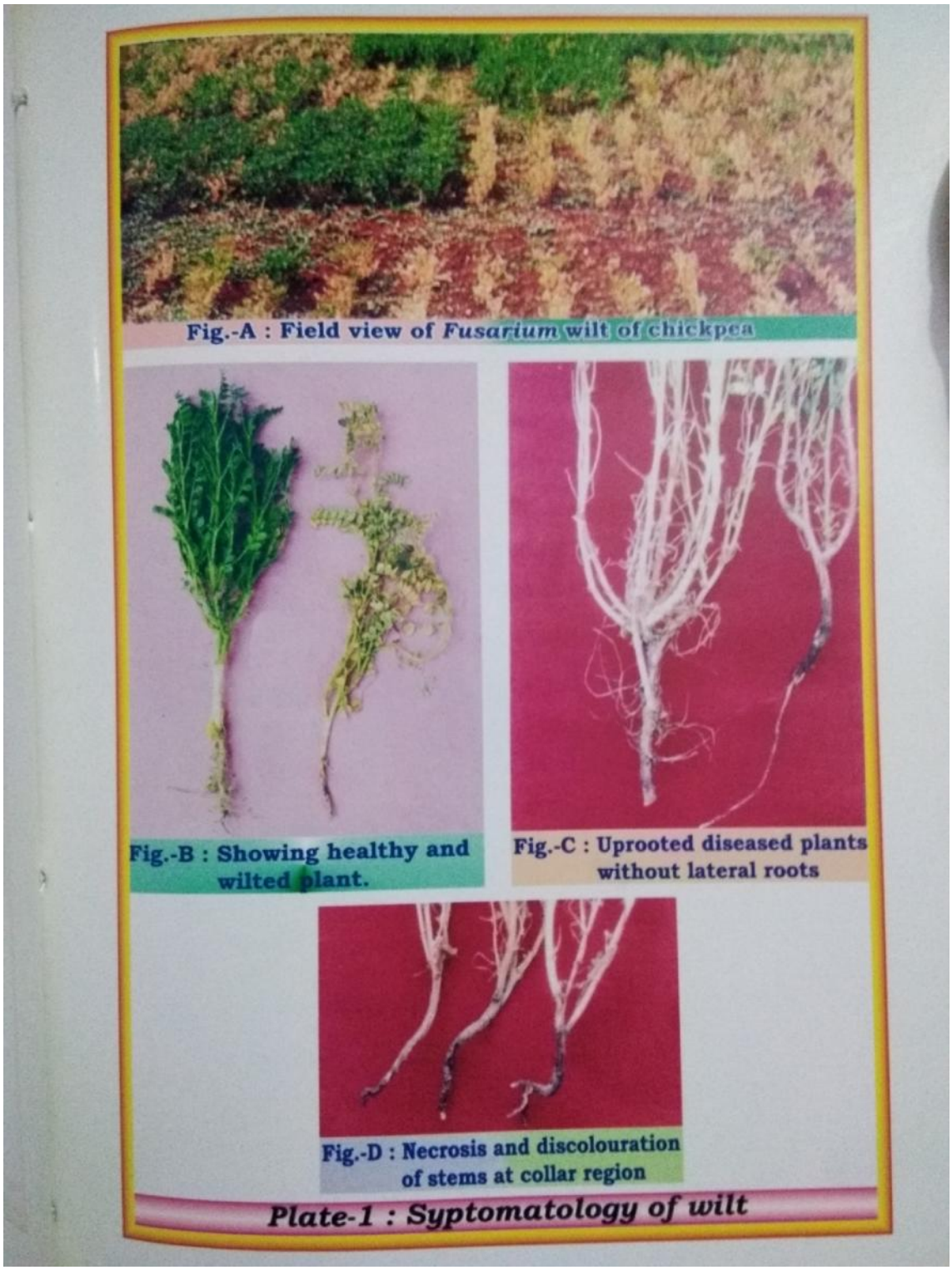
Seedling symptom test followed by serial isolation indicated that the pathogen was transmitted from seed to seedlings within 15 days. It produced drooping of the seedling from the top with browning of rootlets in seedling symptoms test. The pathogen was found systemic as it was recovered from the healthy tissues as well as diseased tissues from roots to the top of the seedlings in serial isolation. The seed transmission was varied from 4.5:1 to 9:1 in different varieties. It was maximum in variety Radhey (4.5:1) and minimum in ICP-1454 (9:1). The ratio between seed coat: cotyledons: embryonal axis was 2.22: 1.44: 1 (indicated by Khune and Patil (1992) in their earlier studies). In the present study 8 fungicides were bio-assayed against the *F. oxysporum* f. sp. *ciceri* by using poison food technique. All 8 fungi toxicant were found significantly effective over control. Of them only two fungicides *viz*., Companion and Bavistin T completely inhibited the growth of the pathogen. The remaining 6 fungicides although inhibited the fungal growth of the pathogen to varying degrees, but failed to inhibit fungal growth completely and considered as partially effective fungicides. Among the partially fungicides Bavistin was most effective followed by Vitavax, Thiram, Mancozeb, Cuman L, and Captan. The above results are in concurrence to the finding of Gupta *et al.* (1988), who reported that Bavistin minimized the growth of the wilt pathogen. The disease was controlled by Carbendazim (50 WP and 25 DS) and Thiram alone and in combination (Singh *et al.,* 1992; Sugha *et al.,* 1995; Gupta *et al.*, 1997).

Conclusion

Symptomatology of the disease

The field symptoms of wilt were seen usually in patches of dead seedlings or adult plants seen usually in patches. The disease can affect the crop at any stage. The first symptom of the disease in the field was drooping of plants followed by sudden death. The leaves also turn yellow and drop off prematurely (**Plate-1**).

In the collar region of the wilted plants necrosis and discoloration of the external tissues can be seen. The diseased plants can be pulled out from the soil more easily than the healthy one. The roots become weak due to disease and when a diseased plant was pulled out, most of the lateral roots remain in the soil. Transverse section of the basal stem or the root reveals masses of fungal hyphae in the vascular bundles and discoloration of vascular cells under the microscope.



Isolation, purification, identification and pathogenicity test of the causal organism

A. Isolation and purification of the pathogen: Naturally affected (wilted) plants of Chickpea collected from wilt sick plot at Legume Section, C. S. Azad University of Agriculture and Technology, Kanpur during November-December, 2002, were examined in the laboratory for the presence of pathogen. The pathogen was readily isolated on 2 per cent Potato Dextrose Agar medium as described under Materials and Methods. The culture of the fungus was later purified by transferring individual hypal tips on 2 per cent Potato Dextrose Agar in culture tubes.

B. Identification of the pathogen: The pathogen was identified on the basis of following morphological and growth habit characters.

(I) Colony characteristic: On Potato Dextrose Agar medium the colonies were white initially, radiating are early stage of growth with wine-red pigmentation of medium in later stages which was clearly visible from the bottom side of the plate.

(II) Mycelium: On Potato Dextrose Agar medium the mycelium was profusely benched, creeping, hyaline, cylindrical, septate and measuring 3.20 - 4.6 µm in width.

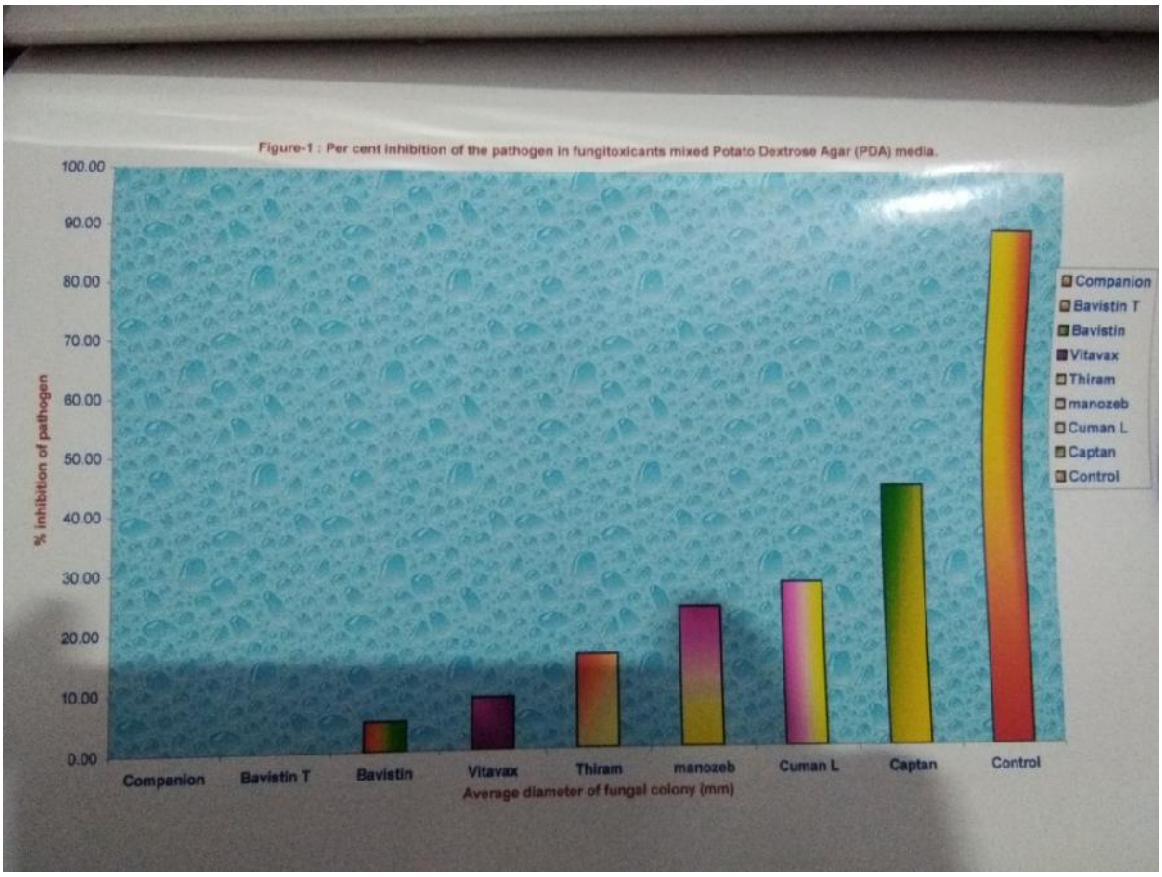
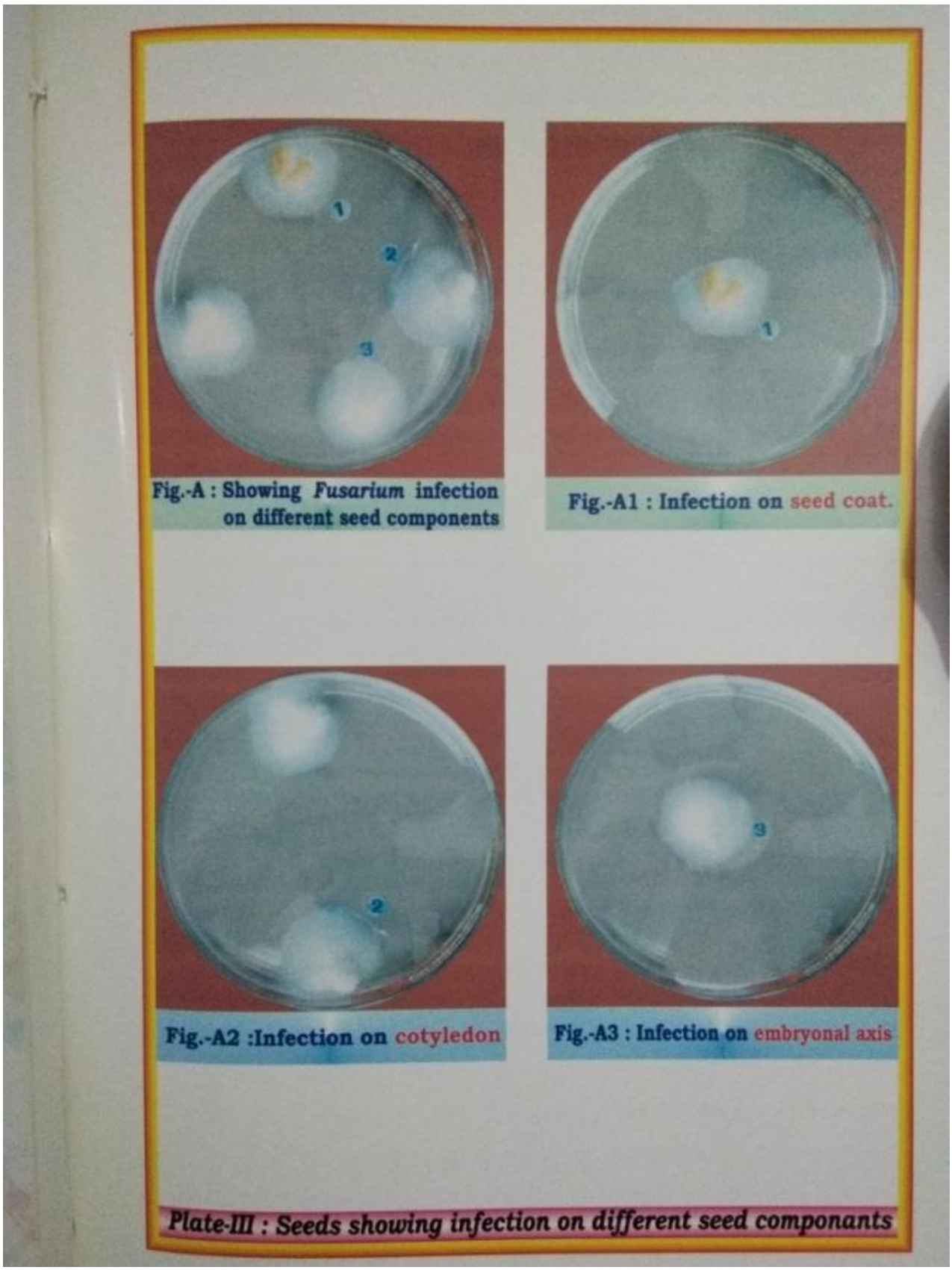


Figure-1 : Per cent inhibition of the pathogen in fungitoxicants mixed Potato Dextrose Agar (PDA) media.
% inhibition of pathogen
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Companion
Bavistin T
Bavistin
Vitavax
Thiram
manozeb
Cuman L
Captan
Control
Average diameter of fungal colony (mm)


Fig.-1 : Seed treated with $HgCl_2$
Fig.-2 : Seed treated with NaOCl
Fig.-3 : Seed with out any treatment
Plate-II : Detection of seed infection by Agar Plate Method



II) Sporodochia: The fungus produces white to light orange sporodochia on PDA. Sporodochia if present with completely covered aerial mycelium.

(III) Microconidia: The microconidia were hyaline, single celled, oval to cylindrical, straight to slightly curved and measuring 2.5 - 3.5 X 5 - 11 µm in size.

(IV) Macroconidia: The macroconidia were sparse and produced on branched macro conidiophores. They were fucoids with pointed ends, hyaline septate (3-4 septate) and measuring 3.0-4.5 X 20-55 µm in size.

(V) Chlamydospores: The Chlamydospores were usually intercalary and one produced singly or in pairs. They were globes to sub-globes, thick walled and smooth surfaced. Chlamydospores like swellings are often seen on the hyphae and measuring 7.0 -8 X 3.5 - 5.0 µm.

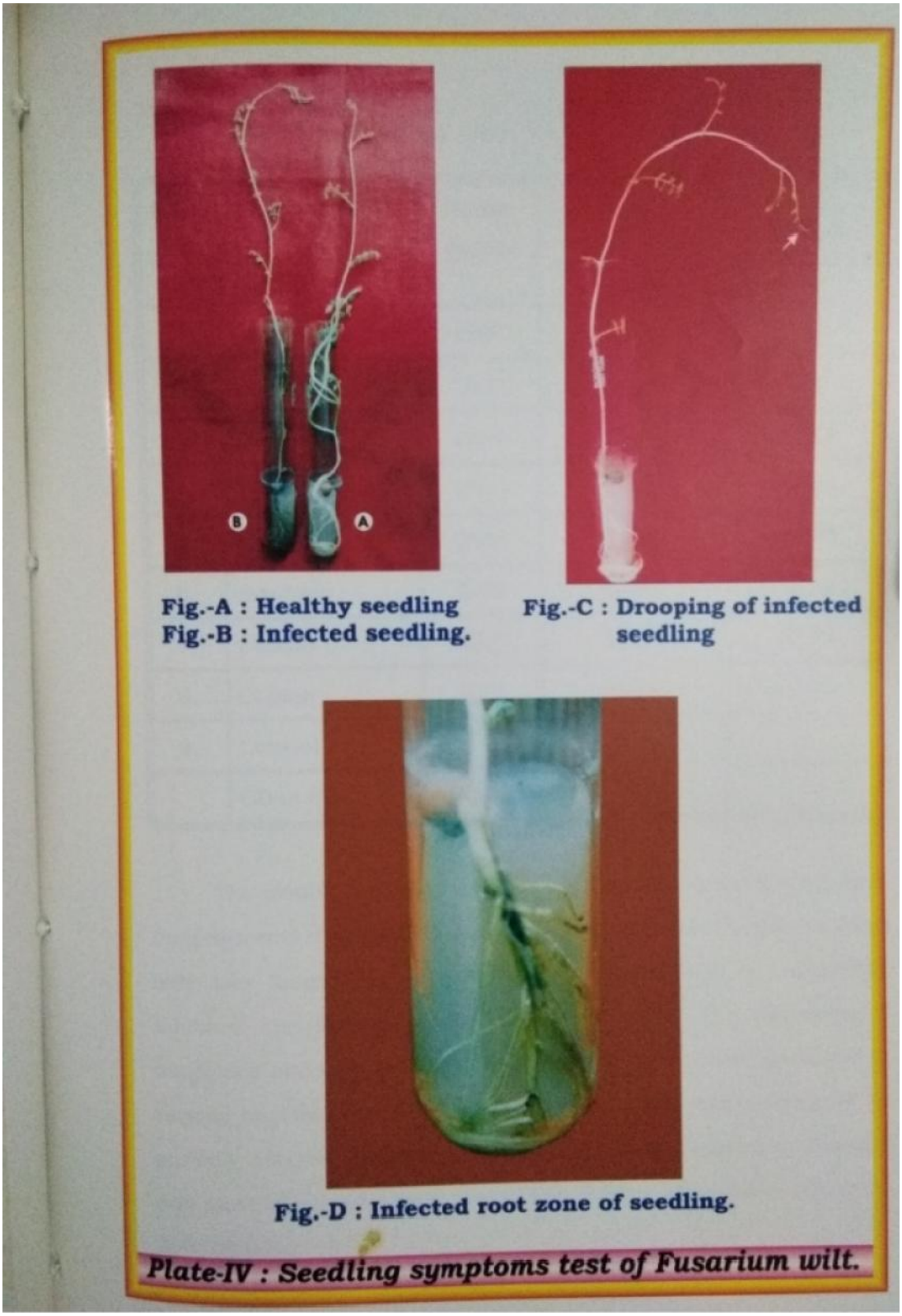


Table 2. Effect of wilt pathogen on the seed, seedling and plant of chickpea variety Radhey

Treatments	No. of seeds germinated	No. of seedlings	No. of plants wilted		
			Seedling stage	Adult stage	Total wilting
Partially sterilized soil inoculated with *F. oxysporum* f. sp. *ciceri*	75	54	20	14	34
Partially sterilized soil not inoculated with the fungus (control)	85	85	0	0	0

Table 3. Per cent seed infection of *F. oxysporum* f. sp. *ciceri* in chickpea cultivars in Standard blotter method and Agar plate method

S.No.	Sample	Standard blotter method			Agar plate method		
		Untreated	Protected with		Untreated	Protected with	
			NaOCl	HgCl2		NaOCl	HgCl2
Susceptible cultivars							
1.	ICP-1454	20	15	6	20	14	5
2.	Radhey	19	13	5	18	12	5
3.	ICC-1876	17	12	3	12	7	4
Resistant cultivar							
4.	Avarodhi	2	1	0	3	0	0
5.	ICC-203	0	0	0	0	0	0
6.	ICCV-32	0	0	0	0	0	0

Table 4. Per cent infection of *F. oxysporum* f. sp. *ciceri* in different seed components (Total number of seeds tested: 100)

Seed sample	Seeds showing infection in blotter test	Seed components		
		Seed coat	Cotyledon	Embryonal axis
ICP-1454	15	15	11	6
Radhey	13	13	8	8
ICC-1876	12	12	7	4
Average	13.33	13.33	8.67	6

Table 5. Showing survivability of *F. oxysporum* f. sp. *ciceri* in seed during off- season

Varieties	ICP-1454	Radhey	ICC-1876
May 2003	+	+	+
June 2003	+	+	+
July 2003	+	+	+
August 2003	+	+	+
September 2003	+	+	+
October 2003	+	+	+
November 2003	+	+	+
December 2003	+	+	+
January 2004	-	+	-
February 2004	-	+	-
March 2004	-	+	-
April 2004	-	-	-

Table 6. Transmission of the pathogen from seed to seedling (seedling symptom test)

Seed sample /varieties	Per cent infection of *F. oxysporum* f. sp. *ciceri*			
	No. of seeds shown	Seeds showing infection	Seedling showing disease symptom	Seed infection seed transmission
ICP-1454	100	18	2	9 : 1
Radhey	100	18	4	4.5 : 1
ICC-1876	100	12	2	6 : 1
Average	100	16	2.67	6 : 1

Table 7. Per cent inhibition of the pathogen in fungi toxicants mixed potato dextrose agar (PDA) media

S.No.	Fungi toxicants	Doses (ppm)	Average diameter of fungal colony (mm)	Per cent inhibition over control
1.	Companion	1500	0.00	100
2.	Bavistin T	1500	0.00	100
3.	Bavistin	2000	5.00	94.40
4.	Vitavax	2000	8.67	90.36
5.	Thiram	2500	15.67	82.59
6.	Mancozeb	2500	23.67	73.70
7.	Cuman L	2500	28.00	68.89
8.	Captan	3000	45.00	50.00
9.	Control	-	90.00	-
	CD at 5%		4.61	

C. Pathogenicity test: In order to ascertain the pathogenic ability of the isolate of *Fusarium oxysporum* f.sp. *ciceri*, the pathogenicity test was conducted on chickpea variety Radhey as described under Materials and Methods, The results on pathogenicity test are given in **Table—2**. The results presented in table-2 showed that the fungus had adverse effect on the seed germination and seedling emergence. The seed germination was reduced by 11.8%. Out of 75 germinated seeds, 54 seedlings were emerged in pots containing pathogen mixed partially sterilized soil. The reduction in germination and seeding emergence was due do rotting of seeds and pre-emergence rotting of germinated seeds.

The initial symptoms of wilting start appearing after 15 days of sowing in the seedlings as drying of seedling from the tip and 20 plants seedlings got infected within two months where as the wilting recorded at the time of adult stage was 14 plants only. The fine roots of the wilted plants were mainly affected and bear dark blacks streak beneath of their bark. Such roots were brittle and easily broken when touched. The brown colour mycelium of the pathogen choked the xylem of primary roots, which resulted in to the drying of whole seedling and plant. The fungus was re-isolated from the diseased tissue and was found same as after comparing with the original one and thus proving the koch's postulate (1882).

To assess the extent of *Fusarium oxysporum* f. sp. *ciceri* infection in the seed of different varieties of chickpea

In order to ascertain the extent of *F. oxysporum* f. sp. *ciceri* infection in seeds, seeds of six, varieties *viz.*, ICP-1454, ICC-1876, Radhey, ICC-203, Avarodhi and ICCV- 32 were tested by Standard blotter method and Agar plate method as described under Materials and Methods. The data on per cent seeds infection of *Fusarium oxysporum* f. sp. *ciceri* in different chickpea cultivars in standard blotter method and agar plate methods are presented in **Table-3**. The results in **Table-3** show that the pathogen *(F. oxysporum* f. sp. *ciceri*) was present in seeds (12-20%) of the varieties of chickpea, which are happened to be susceptible to thus field. The seeds of resistant variety Avarodhi also carried the pathogen to the extent of 3 per cent. The seeds of three resistant varieties accept Avarodhi did not carry the pathogen. Standard blotter method had an edge over agar plate method (**Plate-II**) so for as the detection of the pathogen was concerned. The pre-treatment of the seed with the sodium hypochlorite for 10 minutes and 0.1 per cent mercuric chloride for 1 minute reduced the recovery of the pathogen from the infected seeds. The average recovery percentage from sodium hypochlorite and mercuric chloride pre-treated seeds were 12.17 and 4.67 per cent, respective. These were some deference's in the recovery of seed infection in the varieties .It was maximum in the variety ICP-1454 and minimum in the resistant variety Avarodhi whereas two resistant varieties ICC-203, and ICCV-32 did not carry the pathogen.

Location of the pathogen in the different seed component of chickpea

To see the exact location of the pathogen in different seed components, the seeds of three varieties of chickpea *viz.*, ICP-1454, Radhey and ICC-1876 were collected from post padding wilted plants. The study was conducted by using component platting method as described under Materials and Methods. The data on per cent infection in different seed components are presented in **Table-4.** Data presented in the **Table-4**, and (**Plate-III**), reveal that the pathogen was present in all the components of infected seeds. It was present in the seed coat of all the infected seeds and then entered into the deep tissues of cotyledons and embryonal axis to the extent of 65.04 and 45.01 per cent, respectively. The infection in the seed components was varied with the varieties. It was maximum in ICP-1454 and minimum in ICC-1876. The foregoing experiment indicated that the pathogen was carried with the chickpea seeds externally on its surface and internally in the tissues of seed coat, cotyledons and embryonal axis in the ratio of 2.22: 1.44: 1.

To study of the survival of the pathogen in the seed

In order to see the survival of *F. oxysporum* f. sp. *ciceri* in the infected seeds of varieties *viz*., ICP-1454, Radhey, and ICC-1876 were collected from particularly wilted plant. The seeds were stored in the paper bags under ambient condition from May 2003 to April 2004. The seeds were tested for the presence of the pathogen at monthly intervals as described under Materials and Methods. The data on the survival of the pathogen are presented in the **Table-5**. Results in **Table-5** shows that the pathogen survived in the seeds of all varieties from 8 to 11 months. There was prolonged survival of the pathogen recorded in the Variety Radhey (11 months), while in ICP-1454 and ICC-1876 (8 months). The results indicated that the pathogen was able to survive in the seeds during off season.

To study the transmission of the pathogen from seed to seedling.

(A) Seedling symptom test

The transmission of the pathogen from infected seed to seedling of varieties ICP-1454, Radhey and ICC-1876 was studied by using seedling symptom test and serial isolation method a described under Materials and Methods. The results are presented in the **Table-6.** The results presented in **Table-6** revealed that the pathogen was transmitted from seed to seedling within 15 days. It produced drooping of the seedlings from the top with browning of rootlets in seedling symptom test. The pathogen was found systemic as it was recorded from the healthy tissues as well as diseased tissues from roots to the top of the seedling in serial isolation tests. The average ratio of seed infection and seed transmission was varied from 9: 1 to 4.5: 1 in different varieties. It was maximum in variety Radhey (4.5: 1) and minimum in ICP-1454 (9: 1).

The disease syndrome as observed in seedling symptom test indicated that the seeds in culture tubes started showing the browning of the lateral roots (Primary roots) within 10 days after sowing. In next two days secondary roots of the affected seedlings become brown (**Plate-IV**). The whole of the root system of the affected seedlings was covered with the fungus within 15 days of showings and become brown. There after the yellowing of the leaves with drooping of the seedling started in next 2-5 day. The affected seedling then collapsed and dried out. For confirming the presence of *F. oxysporum* f. sp. *ciceri* in affected seedlings, the roots and stem pieces were placed on potato dextrose agar (PDA) medium in Petri-plates and incubated for 7 days at 25 ± 10C. After 7 days of incubation typical growth of the fungus was a seed that was later identified as *F. oxysporum* f. sp. *ciceri*.

Management of the disease by chemical method

In order to screen the fungicides six fungicide *viz*., Bavistin 50 WP, Thiram75 DS, Vitavax, Cuman L. Mancozeb, Captan, and two combination of fungicides *viz.*, Companion (Carbendazim 12% + Mancozeb 63%) and Bavistin T (Carbendazim + Thiram in ratio of 1:2) in ratio one were assessed for their efficacy against *F. oxysporum* f. sp. *ciceri* through laboratory bio-assay, using poison food technique as described under Materials and Methods.

The growth of the fungus in various treatments was measured diagonally and the average diameter of the colony in each Petri-plate was recorded. The percent inhibition over control was calculated for each treatment and the results are presented in **Table-7**. The results presented in **Table-7** and graph I reveal that all the 8 fungi toxicants' were found significantly superior over control. Of them only two fungicides *viz*., companion and Bavistin T. completely inhibited the fungal growth of the pathogen. The remaining 6 fungicides although inhibited the fungal growth of the pathogen to varying degrees, but failed to inhibit completely and considered as partially affective fungicides, among the partially fungicides Bavistin was most effective followed by Thiram, Mancozeb, Cuman L. and Captan.

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