Studies on seed-borne pathogens of chickpea and their control

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MASTER OF SCIENCE

In

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(Plant Pathology)

By

VANDANA CHADAR
(Enrollment Number-160318003)

Department of Plant Pathology
College of Agriculture, Tikamgarh 472001
Jawaharlal Nehru Krishi Vishwa Vidyalaya
Jabalpur, - 482004 (M.P.)

2018

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*This is to certify that the thesis entitled, "***Studies on seed-borne pathogens of chickpea and their control***" submitted in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE in AGRICULTURE (Plant Pathology)** *of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by* **Ms. Vandana Chadar** *under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.*

All the assistance and help received during the course of the investigation has been acknowledged by him.

Place: Tikamgarh

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Member	**(Dr. A. K. Shrivastava)**	----------

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Date: Chairman of the Advisory Committee

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Committee	Name	Signature
Chairman	**(Mrs. Shraddha Karcho)**	----------
Member	**(Dr. R. K. Prajapati)**	----------
Member	**(Dr. M. K. Nayak)**	----------
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I, **Vandana Chadar** D/o **Sh. Deendayal Chadar** certify the work embodied in thesis entitled, **"Studies on seed-borne pathogens of chickpea and their control."** is my own first hand bonafide work carried out by me under the guidance of **Mrs. Shraddha Karcho, Assistant Professor,** Department of Plant Pathology, College of Agriculture, Tikamgarh (M.P.) during 2017-18.

The matter embodied in the thesis has not been submitted for the award of any degree/diploma. Due credit has been made to all the assistance and help.

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Place: Tikamgarh

Dated / /2018 Vandana Chadar

List of Contents

List of Tables

List of Figures

List of Plates

List of Appendices

List of Abbreviations

Words		Abbreviation/symbol
Completely Randomized Block Design	:	CRBD
Co-workers	:	*et al.*
viz.,	:	Namely
Critical difference	:	CD
Degree Celsius	:	°C
Gram	:	G
Jawaharlal Nehru Krishi VishwaVidyalaya	:	J.N.K.V.V
Kilogram	:	Kg
Milimetre	:	Mm
Significant	:	S
Per cent	:	%
Per hectare	:	ha^{-1}
JG	:	Jawahar Gram
Spicies	:	spp. or sp.
Standard error of means	:	Sem±
Temperature	:	Temp.
Wettable Powder	:	WP
Emulsifiable Concentrate	:	EC
Soluble Concentration	:	SC
PDA	:	Potato Dextrose Agar
At the rate	:	@
Milileter		Ml

INTRODUCTION

Pulses have special significance in the dietary of the predominantly vegetarian population of India as they contain more protein on dry weight basis which is 2 to 3 times more than in cereals. Chickpea (*Cicer arietinum*) is one of the most important pulse crops and belongs to the family Fabaceae. There are two distinct types of cultivated chickpea: Desi and Kabuli. Kabuli chickpea is considered more economically important as it receives higher market price than desi.

Nutritionally a row chickpeas is very rich as it contains 17.21% protein, 62% carbohydrates, good amount of fat, besides it is rich source of Ca, Fe and vitamin-C (green stage) and vitamin–B. Its leaves consist of malic acid and citric acid which are very useful for stomach ailments and for blood purification (Padmaja *et al*., 2015). Among the major pulse crops, chickpea contributes 42 to 47 % of total pulse production. It also plays a major role in management of soil fertility particularly in dry lands.

Chickpea is cultivated mostly in the Mediterranean basin, Central and South Asia, East Africa, America and more recently in Australia. The main producers are India, Australia and Pakistan, contributing global production of 67.32%, 6.19% and 5.72%, respectively (FAOSTAT, 2016).

In Madhya Pradesh, the chickpea production was 22.97 lakh tonnes from an area of 26.21 lakh ha. with an average productivity of 877 kg ha^{-1} during 2015-16 (Anon., 2016). In Tikamgarh district, the annual production was 240.26 tonnes from 20.26 ha with an average productivity 1200 $kgha^{-1}$ (Anon., 2017).

Chickpea cultivation is often subjected to significant yield losses due to insects and diseases ranging from 5-10% in temperate and 50-100% in tropical regions (Van Emden *et* al., 1988). Currently chickpea is affected by 172 pathogens of which 67 fungi, 3 bacteria, 22 viruses and mycoplasma, and 80 nematodes reported from 55 countries. Maximum

number of pathogens infecting chickpea (89) had been reported from India while in other countries, it varied from 1 to 40 (Nene *et al.*, 1996).

Important fungal diseases of chickpea and their causal organisms are Dry root rot (*Rhizoctonia bataticola*), Furarium wilt (*Fusarium oxysporum f. sp. ciceri*), Seedling/ seed rot (*Aspergillus niger*), Ear rot of maize (*Penicillium sp.)*, soft rot of vegetables (*Rhizopus sp.)*

Despite of different diseases, Fusarium wilt is most important disease of chickpea causes severe damage. Although the disease is wide spread in the chickpea growing areas of the world and most prevalent in the Mediterranean Basin and the Indian subcontinent (Jalali and Chand, 1992). The dry root rot (*R. bataticola)* is an important plant pathogen with worldwide distribution and wide host range and with variable characteristics. The species *R. bataticola* is a pathogen of over 290 plant species (Dhingra and Sinclair, 1993).

To increase the production of chickpea qualitatively and quantitatively, farmers requires healthy and quality seeds with high percentage of seed germination and purity. Hence, it is imperative that seeds must be tested before they are sown in the field. Another adverse effect of seed-borne pathogen is that it will contaminate the areas which were disease free previously. So, it necessitates the eradication of seed-borne inoculums through various seed treatment and through enforcement of proper domestic and international quarantine act and procedures.

In view of its importance and significance of seed-borne diseases of chickpea, "**Studies on seed-borne pathogens of chickpea and their control**" was carried out with the following objectives:

1). Survey and collection of seed samples from chickpea growing area of district Tikamgarh (M.P.).
2). Detection of seed-borne mycoflora from collected diseased chickpea seeds.
3). Evaluation of fungicides, bio-agents and bio-pesticides against major seed-borne pathogens of chickpea seed *in-vitro*.

REVIEW OF LITERATURE

A brief review on available literature pertaining to seed-borne pathogens of chickpea and their control is divided into the following headings and subheadings as under:

2.1 Occurrence and distribution.

2.2 Seed mycoflora of chickpea.

2.3 Seed health testing methods.

2.4 Evaluation of fungicides, bio-agents and bio-pesticides against seed-borne pathogens of chickpea seed *in-vitro.*

2.1 OCCURENCE AND DISTRIBUTION:-

.Gupta *et al.* (1983) observed disease incidence of root rot ranging from 3.5-20.6 percent in 30 villages of northern Madhya Pradesh.

Prajapati *et al*. (2003) reported the prevalence and incidence of dry root rot of chickpea from surveyed 20 locations of Uttar Pradesh and Uttaranchal. He reported that the dry root rot was prevalent in all the area were surveyed and the range of incidence was 12 to 35 per cent. The maximum incidence was recorded at Banda district of up and minimum at Pantnagar of Uttaranchal. The bold seeded varieties were found more prone to the dry root rot than small seeded.

Gurha and Trivedi (2008) surveyed chickpea fields in Shimoga, Raichur and Bangalore districts in Karnataka for the prevalence of dry root rot. *R. bataticola* was the predominant pathogen infecting 60 to 70% of the plants in the fields of Gulbarga, Manchalapur, Eklaspur, Raichur and Tengri.

Aghakhani and Dubey (2009) found twenty three isolates of *R. bataticola* causing dry root rot of chickpea collected from 10 different major chickpea growing states (Karnataka, Haryana, New Delhi, Punjab, Uttar Pradesh, Maharashtra, Jharkhand, Rajasthan, Madhya Pradesh and Chattisgarh) of India were highly variable in their morphological characters. The sclerotium formed in different isolates was dark brown to

black and size varied from 40 - 600 µm. The isolates from Bangalore (Rb1) and Faridkot (Rb 5) produced the largest size sclerotia ranged from 200 – 600 µm and 100 – 400 µm respectively. Dumka (Rb19) isolate produced small sized sclerotia which ranged from 43.9- 81 µm. They observed most virulent isolate (Rb1 from Bangalore, Karnataka) was fast growing and produced largest sclerotia. The isolates were highly variable in virulence.

Manjunatha and Naik (2011) survey was conducted during november to december, 2008 in the farmers' fields at Raichur, Gulbarga and Bidar districts in Karnataka. The maximum dry root rot incidence in chickpea was noticed in Gulbarga district (9.8%) followed by Raichur (7.6%) and the least (6.18 %) in Bidar. The overall incidence of dry root rot ranged from1-19% across the districts. This was mainly attributed due to variation in soil type (black and red soil) and cultivars grown (Annegiri and JG 11) under rain fed condition.

Khan *et al.* (2012) surveyed for the occurrence and severity of chickpea dry root rot was made during crop season 2010-11 in Jammu and Kashmir. The highest incidence was up to 40% in Shangus and lowest up to 4.11% in Naina of Jammu and Kashmir. The incidence of dry root rot in chickpea caused by *R. bataticola* was observed in late- October to mid-november. The intensity of the disease was high in the month of february and march during late flowering and podding stage.

Ghosh *et al.* (2013) survey was conducted in 2010-2011 *rabi* cropping season to obtain information on the distribution and incidence of chickpea diseases with respect to soil type, cultivar used, seed treatment in Central and Southern parts of India (Andhra Pradesh, Karnataka, Madhya Pradesh and Chhattisgarh). Local cultivars Annigeri (19.23%) followed by Harbora (12.82%) were most frequently grown by the farmers. However, among the improved varieties JG 11, JG 130, JG 16, JG 74 and Jawahar were most commonly grown covering 34.61% area. Dry root rot disease was found in all the sites surveyed where the incidence ranged

from 8.9-10.3% irrespective of cultivar type and location. Disease incidence was lower on improved (9%) as compared to local varieties (14%). Sixty three percent of the farmers practiced seed treatment with fungicides. The disease incidence in seed treated fields ranged from 6.63-12.40% compared with untreated 9.22-20.02%.

Zemouli- Benfreha *et al.* (2014) survey at three agro climatic zones of north–western Algeria through seven sites in year 2005 to 2009. The presence of the fusarium wilt disease was found in all the 50 fields chickpea visited.

Tripathi (2015) survey was made at 30 days of interval from sowing after maturity stage on chickpea growing area of district Tikamgarh and recorded the incidence of fusarium wilt from 04.99 to 26.62.

2.2 SEED MYCOFLORA OF CHICKPEA:-

Neergaard (1977) observed that seeds are the efficient carriers for survival, large scale and long distance spread of pathogens. Infected seeds serve as major source of inoculums for large number of plant pathogens which may infect the seeds and survive as spore or resting structures on or within the seeds.

Haware *et al*. (1978) reported the internal transmission of *F. oxysporum* f. sp. *ciceri* Fungus was found in the hilum region of chickpea (*Cicer arietinum* L.) seeds collected from diseased plants.

Bretag and Mebalds (1987) isolated surface-borne saprophytes *Alternaria alternata, Penicillium spp Aureobasidium pullulans, Stemphylium spp., Cladosporium spp., Rhizopus stolonifer, Vlocladium atrum* and several pathogens like *Ascochyta pisi, Botrytis cinerea, Fusarium oxysporum* and *Phoma medicoginis* from chickpea seed by employing standard blotter method.

Paul (1989) reported the association of *Aspergillus* (12%), *Cladosporium* (56%), *Fusarium* (75.25*%), Penicillium* (7.25%), *Phoma* (25.25%) and *Rhizopus* (12%) with soybean seeds when assayed under

blotter method. The number of colonies of *Aspergillus* and *Rhizopus* were increased by 27 and 64 per cent respectively.

Dwivedi and Shukla (1990) observed the association of 20 fungal species belonging to 12 genera (7 *Aspergillus* spp., 2 *Fusarium* spp. and 2 *Penicillium* spp.) with chickpea seeds. Among which *Acremonium implicatum, Aspergillus melleus, A. tereus, A. wentii, Drechslera spicifera, Bipolaris australiensis, Penicillium puberulum, Epicoccum purpurascens,* and *Scytalidium* sp. were newly recorded.

Mussa and Abdulsalam (1991) isolated *A. alternata* and *F. oxysporum* from two chickpea genotypes CNJ and E14 under laboratory condition.

Brayford (1992) reported the vascular wilt of chickpea caused by *F. oxysporum* f. sp. *ciceri* was first recorded from India. The pathogen was found as soil borne and may also be transmitted by seed.

Haware *et al*. (1996) observed the chickpea Fusarium wilt is a seed and soil borne disease of economic importance and can be isolated from all parts of infected plants including seed as the fungus is systemic.

Demirci *et al*. (1998) reported that the fungi most frequently isolated from infected plants were *F. solani* f. sp. *pisi, R. solani*. *F. oxysporum* f. sp. *ciceri* and *M. phaseolina.*

Rauf (2000) observed the twenty-four seed-borne fungi belonging to different genera using blotter paper method, from 145 seed samples of major legume crops in Pakistan of which, *A. alternata, Ascochyta spp*., *Colletotrichum spp., Fusarium spp.* and *M. phaseolina* were the most frequent and known as common pathogenic fungi in these crops. Highest number of various types of mycoflora was detected in soybean (14 fungal species) and chickpea (13 fungal species) followed by mung bean, pea and lentil seeds.

Singh *et al*. (2005) observed on seven seed samples of chickpea were isolated nine fungal species belonging to eight genera namely, *A. alternata, Aspergillus flavus, A. niger, Curvularia lunata, F. moniliforme, Helminthosporium sativum, Mucor* sp., *Penicillium notatum* and *Rhizopus nigricans.*

Toorray *et al.* (2005) studied the association of seed-borne mycoflora with 75 accessions of chickpea. Seven accessions did not show the association of mycoflora and they showed 100 per cent germination and no post-emergence mortality while eight fungi, *viz., A. niger, A. flavus, Rhizoctonia* sp., *Rhizopus* sp., *Fusarium* sp., *Alternaria* sp., *Curvularia* sp. and *Ascochyta* sp. were isolated from the seeds of 68 accessions.

Dawar *et al.* (2007a) isolated *Absidia glauca, R. solani, Syncephalastrum* sp., *Trichoderma harzianum, F. moniliforme, F. oxysporum, M. phaseolina, R. solani, A. niger, and A. flavus* from 14 chickpea seed samples.

Hussain *et al*. (2007a) isolated fungi *viz.*, *F. moniliforme, A. alternata, Mucor hiemalis, Chaetomium* spp. *Penicillium citrinum, A. niger, A. flavus, A. terreus, Nigrospora* spp., from lentil seeds collected from Punjab area of Pakistan using blotter method and agar plate method.

Chaithra M. (2009) revealed the dominance of *Fusarium* spp. Seed washing technique revealed only the presence of saprophytic fungi like *Aspergillus* sp. Among the different seed health testing methods, 2,4-D blotter method was found to be good for the detection of *Fusarium* spp. in chickpea

Agarwal *et al*. (2011) isolated *A. alternata, Chaetomium spp., P. citrinum, A. niger, A. flavus, Rhizopus nigricans, F. oxysporum* from chickpea, lentil and black gram seeds.

Saroja *et al*. (2011a) recorded 10 species of fungi associated with chickpea seeds viz., *Alternaria sp., A. flavus, A. fumigates, A.*

terreus, A. niger, Curvularia lunata, Fusarium sp., Penicillium chrysogenum, Rhizopus stolonifer and *Trichoderma sp*. following standard blotter paper and agar plate method collected from farmers seed lots.

Patil *et al*. (2012a) isolates the pigeon pea and chickpea seeds using agar plate method and found that untreated seeds the per cent incidence of *A. flavus* (30%) was the highest followed by *A. niger* (25%), *P. notatum* (20%), *Cladosporium herbarum* (18%), where as all other fungi were within the range of (3 to 15 %).

Mailem (2013) reported the ten fungal species *viz*., *F. oxysporum* f. sp. *ciceri*, *A. niger*, *A. flavus*, *Rhizoctonia* sp., *Alternaria* sp., *Penicillium* sp., *Curvularia* sp., *Rhizopus* sp., *Chaetomium* sp., and *Trichoderma* sp., were found associated with seeds of chickpea cultivars. Out of these, occurrence of *F. oxysporum* f. sp. *ciceri* was found predominant in both standard blotter (41.79 %) and agar plate method (32.53 %), while occurrence of *Penicillium* sp. was least (1.49 and 0.60 %).

2.3 SEED HEALTH TESTING METHODS:-

Neergaard (1956) suggested the dipping of blotters in 0.2 per cent 2-4 dichlorophenoxy acetic acid for detecting fungal infection in cabbage seeds since it avoids germination of seeds.

Shakir and Mirza (1994) used standard blotter method and agar plate method to confirm the presence of fungi in different seed parts and found that agar plate method yields a greater number of fungi which includes *A. rabiei, F. oxysporum* and *A. alternata*.

Singh *et al*. (2005) reported that standard blotter method was best method in terms of number of fungal species isolated from chickpea seed followed by agar plate method.

Dawar *et al.* (2007b) reported that blotter paper method showed greater incidence of fungi on different parts of chickpea seeds followed by agar plate and deep-freezing method.

Hussain *et al*. (2007b) observed the per cent infection of untreated and treated seeds of lentil were more in potato dextrose agar method with an average of 52.52 and 20.87% when compared to that of blotter method with an average of 46.96 and 20.4 % respectively.

Ishrat Niaz and Shahnaz Dawar (2009) reported that deep freezing method was the best method for the detection of *Drechslera* spp., *Fusarium* sp. and *Penicillium* spp., while agar plate method was suitable for the detection of *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., and *Rhizopus* spp from maize seeds.

Sarhan (2009) found out a laboratory study to investigate the effect of seed-borne fungi on seed germination and seedling growth of bean, broad bean, chickpea, cowpea, green gram, lentil, and pea. Representative subsamples were examined by regular blotter and agar plating methods. 12 genera of fungi associated with legume seeds were observed *viz.*, *Alternaria* spp., *Aspergillus* spp., *Ascochyta* spp., *Chaetomium* spp., *Cladosporium* spp., *Fusarium* spp., *Geotrichum* spp., *Penicillium* spp., *Pythium* spp., *Rhizoctonia* spp., *Rhizopus* spp. and *Verticillium* spp. Incidence of fungi on legume seeds ranged from 5.0 to 46.2 per cent. *Fusarium* spp. was the most common fungi isolated.

Saroja et al. (2011b) reported that standard blotter paper method was proved to be the best for isolating the seed mycoflora of chickpea.

Patil *et al.* (2012b) evaluated the seed mycoflora was screened by using agar pate method. Untreated seeds the percent inhibition of *Aspergillus flavus* (30 %) was followed by *A. niger (25%), Panicillium notatum (20%), Cladosporium harberum (18%),* where as all other fungi were within the range of (3 to 5%).

Padmaja *et al*. (2015) reported the impact of seed mycoflora was observed maximum on injured seeds. The predominant fungi were *Aspergillus niger* (83%), *F. solani* (79%), *A. flavus* (75%), *A. alternata* (60%) and *A. nidulans* (57%). Minimum seed borne fungi was reported by *R. stolonifer, F. oxysporum*. Fungi like *Cladosporium* sp*., Colletotrichum*

truncatum did not occur on bold seeds. Rest of the fungal species showed their presence on all seed categories. Agar plate method showed more mycoflora as compared to blotter paper method.

2.4 EVALUATION OF FUNGICIDES, BIO-AGENTS AND BIO-PESTICIDE AGEINST MAJOR SEED-BORNE PATHOGENS OF CHICKPEA SEED *IN-VITRO.*

2.4.1: Evaluation of Fungicides

Verma and Vyas (1977) found benomyl, carboxin, thiabendazole and carbendazim as the superior seed dresser against chickpea wilt.

Sharma *et al.* (1978) assessed systemic and contact fungicides in the control of rhizome rot of ginger caused by *F. oxysporum.* They found that the yield of rhizome was increased when they used fungicides. They reported that Bavistin 50 WP was the best fungicides in controlling rhizome rot of ginger, followed by Captain and Difolatan.

Viswakarma and Basu Chaudary (1982) evaluated effect of fungicides against root disease pathogens of gram under *in-vitro* condition. During their study they found that Agrosan GN and RH 893 were found effective against *F. solani* at 5 ppm concentration, but statistically different from benlate, aretan, vitavax – 200, vapam (or) ziram.

Bhat and Srivastava (2003) tested fourteen fungicides (250-1000 ppm; Emisan. Blitox 50 Captaf , Indofil M-45, Bavistin, Benlate, Roko, Saar, Calixin, Tilt. Contaf, Topas, RIL F004 and Contaf 5% SC) and four neem formulations against *F. oxysporum, Pythium aphanidermatum, F. solani, F. moniliforme, Sclerotium rolfsii* and *Fusarium sp.*for inhibition and three *Trichoderma spp*. for compatibility *in-vitro*. Emisan and Saaf (250 ppm) and Triazoles (250-1000 ppm) were highly inhibitory against both pathogens and *Trichoderma spp*. Bavistin and Benlate completely inhibited *Fusarium* and *Trichoderma* even at 250 ppm. Similarly, Captaf, Calixin, RIL F004, Tilt, and Indofil M-45 completely inhibited *P. aphanidermatum* and *S. rolfsii* at the same concentration. Indofl M-45 was

fungistatic against *T. viride*, while showing complete inhibition of *F. solanni. F. oxysporum, P. aphanidermatum* and *S. rolfsii* at 500 ppm**.**

Singh and Jha (2003) evaluated seven fungicides and found that thiram & carbendazim were the most effective in the restricted *F. oxysporum* f. sp. *Ciceri* at 1% concentration.

Podder *et al.* (2004) observed the significant growth inhibition of *F. oxysporum* f. sp. *Ciceri* with all the fungicides at all concentrations. Maximum inhibition (90 mm) was recorded in carbendazim followed by thiophanatemethyl (39 mm) at 50 ppm Propiconazole caused minimum inhibition at all concentrations.

Soma *et al.* (2008) reported that carbendazim and carboxin were highly fungitoxic and showed 100 per cent inhibition in case of *F. oxysporium* at 100 ppm and 200 ppm concentration.

Vinit *et al.* (2010) reported that carbendazim and carboxin completely inhibit the growth of *F. oxysporum* f. sp. *Lentis.*

Masum *et al*. (2009) reported that, five seed treatment practices *viz*., hot water treatment, garlic tablet, Neem leaf extract, BAU- Bio-fungicide and vitavax-200 significantly reduced the total seed-borne fungal infections as well as the population of individual 6-targeted pathogenic fungi *Agrostis tenuis, Bipolaris sorghicola, Botrytis cinerea, Crinum graminicola, Curvularia lunata, F. moniliforme*.

Subhani *et al*. (2011) conducted an experiment to test the fungitoxic effects of six fungicides, *viz*., benomyl, derosal, ridomil, cabrio top, vitavax and prevent at four concentrations, 5, 10, 20 and 50 ppm through poisoned food technique and recorded a significant decrease in mycelial growth of the *F. oxysporum* f. sp. *Ciceri* with an increase in fungicidal concentration. The most effective fungicides in inhibiting the growth of the fungus, in descending order were derosal, benomyl and vitavax as they caused 100, 95.81, 93.80 and 70.96 per cent reduction in mycelial growth, respectively at 5 ppm concentration. The most effective fungicides under *in vitro* conditions were tested in green house grown plants wherein the fungicides

were compared on the basis of mean number of wilted plants at all dosage rates at 39th day of sowing. Derosal and benomyl were the most effective by reducing 100 per cent wilt incidence.

Taskeen–Un-Nisa *et al.* (2011) revealed that all systemic fungicides at different concentrations significantly inhibit the mycelial growth of *F. oxysporum.* However, the hexaconozole at highest concentration (1000 ppm) caused highest reduction of mycelail growth (8.80 mm) followed by carbendazim (9.40 mm), bitertanol (18.60 mm) and myclobutanil (20 mm) at the same concentration. It was also observed from the study that amongst the non-systemic fungicides, mancozeb was found most effective (14.20 mm) in reducing mycelia growth of the fungi followed by captan (20.00 mm) and zineb (22.00 mm).

Sultana and Ghaffar (2013) reported that the complete inhibition of colony growth of *F. oxysoprum* was observed where fungicides *viz*., Aliette, Benlate and Carbendazim @ 100 ppm were used whereas Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the colony growth @ 1000 ppm.

Somu *et al.* (2014) evaluated six fungicides in *in vitro* against *F. oxysporum* f.sp. *cubense,* carbendazim, carboxin, propiconazole and benomyl showed total inhibiton of the fungal growth at the concentrations of 500, 1000 and 2000 ppm. However, difenconazole showed total inhibition of the fungal growth at 2000 ppm concentration

Bashar *et al. (*2014a) observed the highest inhibition of growth of *F. solani* with Vitavax 200B (97.87%), which was followed by Agridazim 50 WP (97.23%), Cozeb 80 WP (96.81%), Sunvit 50 WP (96.81%) and Newban 72 WP (94.89%). In case of *F. oxysporum*, Vitavax caused the maximum inhibition (98.73%). Agridazim 50 wp, cozeb 80 WP, Newban 72 WP and Sunvit 50 WP were responsible for 98.61, 95.95, 96.46 and 91.14% inhibition of *F. oxysporum,* respectively at 500 ppm concentration.

Bana *et al.* (2017) revealed the Maximum inhibition per cent was recorded on Carbendazim (T1-99.44%) which was statistically superior on

Mancozeb (T3- 98.77%) and T3 statistically superior on Calcium chloride (T2-81.33%).

Bashir *et al.* (2017) observed the interaction between treatments and concentration (T × C) exhibited maximum colony growth of all treatments (Carbendazim, Benomyl, Topsin-M, Difenoconazole, Nativo, and Alliete), i.e., 0.87, 1.23, 1.73, 2.20, 2.53, and 2.93 cm at 300 ppm as compared to 500 and 700 ppm concentrations, respectively for management of *F. oxysporium* f. spp. *capsici.*

Rakesh *et al.* (2017) reported the efficacy of six fungicides was tested *in vitro* by poisoned food technique. All the fungicides Carbendazim, Mancozeb, Thiram, Tebuconazole, Carbendazim + Mancozeb and Vitavax + Thiram Recommended Dosages (ppm) 1000, 2500, 3000, 1000, 2000 an 2000 respectively were effective in inhibiting mycelial growth of *F. oxysporum* f. sp. *ricini* to varying degrees. Significant difference was observed among the fungicides in inhibiting the mycelial growth of the pathogen.

2.4.2: BIOAGENTS (*Trichoderma viride and Pseudomonas* spp.):-

Bharadwaj and Gupta (1987) observed in in vitro tests using *Trichoderma viride, T. harzianum* and *T. hamatum against Pseudomonas aphanidermatum, F. equiseti* and *F. solani* showed that these antagonists were inhibitory to the pathogens.

Ho-Seong *et al.* (1991) reported the antifungal activity of antagonistic bacterium *Pseudomonas stutzeri* against *F. solani* isolated from a ginseng rhizosphere. In several biochemical tests with culture filtrates of *P. stutzeri* YPL-1 and in mutational analysis of antifungal activities of reinforced or defective mutants, they found that anti-*F. solani* mechanism of the bacterium may involve a lytic enzyme rather than a toxic substance (or) antibiotic. *P. stutzeri* YPL-1 produced extracellular chitinase and laminarinase when grown on different polymers such as chitin, laminarin (or) *F. solani* mycelium. These lytic extra cellular enzymes

markedly inhibited mycelial growth rather than spore germination and also caused lysis of *F. solani* mycelia and germ tubes.

Rangeshwaran and Prasad (2000) isolated 300 rhizospheric bacteria from different regions of Karnataka and screened for *in vitro* antagonism in dual culture against five fungal pathogens *viz*., *B. cineria, M. phaseolina, S. rolfsii, R. solani* and *F. oxysporum* f. sp. *ciceri*. Four potential antagonists *viz., P. putida* (PDBCAB 19), *P. fluorescens* (PDBCAB 2), *P. fluorescens* (PDBCAB 29) and *P. fluorescens* (PDBCAB 30) were identified and their root colonizing ability was tested. *M. phaseolina, R. solani* and *F. oxysporum* f. sp. *ciceri* were targeted by four selected antagonists under green house conditions and the maximum plant stand (100 per cent) was observed with *P. fluorescens* (PDBCAB 29 and 30) treated pots for *M. phaseolina, R. solani. P. putida* (PDBCAB 19) and *P. fluorescens* (PDBCAB 30) were able to fully control *F. oxysporum* f. sp. *ciceri*.

Sonawane and Pawar (2001) conducted an experiment to study the antagonistic effect of *T. viride, T. harzianum, T. hamatum* and *Aspergillus awamori* against *F. oxysporum* f. sp. *ciceri in vitro* by adopting the dual culture technique in which *T. harzianum* was very effective in controlling vegetative growth of the pathogen followed by *T. hamatum*. Relatively, an integrated management of chickpea wilt was studied by treating the seeds with bioagents and observed that *T. harzianum* was the most effective treatment followed by bavistin and garlic extract.

Dhedhi *et al*. (2002) isolated bacteria (*B. subtilis* B1 and B2; *Bacillus* sp. B3, B4, and B5), actinomycetes (*S. griseus* S1, *Streptomyces* sp. S2, S3, and S4) and fungi (*P. funiculosum*, *P. pinophilum*, *T. harzianum*, and *T. viride*) from the soil which were antagonistic to *F. oxysporum* f. sp. *ciceri* in dual culture. All the antagonists resulted in reduced growth of the pathogen and also the seeds treated with a culture suspension of antagonists reduced the pre- and post-emergence losses in

sterilized and unsterilized soil infested with the test fungus compared to the control (40 per cent).

Mujeebur *et al*. (2004) observed the effect of treating seeds of chickpea cv. BG 256 with commercial formulations (2 g/kg seed) of *T. harzianum* and *P. fluorescens,* singly and jointly, to control wilt caused by *F. oxysporum* f. sp. *Ciceri* under field condition. On untreated control plants, the wilt fungus caused the characteristic symptoms of wilt and significantly decreased dry weight and the yield of chickpea by 20 and 15 per cent, respectively. On chickpea without wilt, treatment with *P. fluorescens* improved the yield by 36 per cent and *T. harzianum* + *P. fluorescens* by 25 per cent. Both biofungicides suppressed wilt severity, the most effective being *T. harzianum* + *P. fluorescens*.

Podder *et al. (*2004b) observed *T. harzianum* (TH-1) isolate caused 43.2% mean growth inhibition of *F. oxysporum* f. sp. *Ciceri* followed by TH-2 which caused 31% growth inhibition.

Mahajan (2007) investigated the antagonistic effect of 13 bacterial isolates by employing dual culture technique on media. Results indicated that all the test organisms inhibited growth of *Fusarium udum* as compared to control.

Nikam *et al.* (2007) observed the *In-vitro* evaluation of *Trichoderma* spp. *Against F. oxysporium* f. sp*. ciceri* revealed that positive cumulated effect of *T. viride* + *T. harzianum* + *T. hamatum* in respect to the percent inhibition of the test fungus.

Sunil et al. (2007) evaluated 10 isolates belonging to three species of *Trichoderma (T. viride, T. harzianum and T. virens*) against four different races of *F. oxysporum* f. sp. *ciceri* commonly prevalent in India Dharwad (Race-1), Kanpur (Race-2), Ludhiana (Race- 3) and Delhi (Race-4)).

Sunita and Kurundkar (2007) evaluated the efficacy of *Trichoderma* isolates against *F. oxysporum f. sp. ciceri* under laboratory condition by employing dual culture technique on potato dextrose agar. Results

indicated that, in general *Trichoderma* isolate inhibited growth of the pathogen.

El-Mohamedy and Abd El-Baky (2008) evaluated the efficacy of different types of seed treatments i.e., bio-priming, seed coating with bio-control agents (*T. harzianum, Bacillus subtilis* and *P. fluorescens*) seed priming and seed dressing with these antagonistic micro-organisms enhanced their effectiveness in control of root rot disease incidence compared to other treatments.

Jayalakshmi et al. (2009) investigated the induction of plant defense response against *F. oxysporum* f. sp*. ciceri* by inoculating the roots of chickpea cv. JG 62 with the bio-control agent *T. harzianum* L1.

Rajput *et al.* (2010) evaluated biocontrol agents *viz*., *T. harzianum*, *T. viride*, *P. fluorescens* and *B. subtilis* against *F. oxysporum* f. sp. *ciceri*, *R. bataticola* and *S. rolfsii* under dual culture and found that hyphal growth of pathogens was inhibited at zone of contact which was maximum in *F. oxysporum* f. sp. *ciceri* (65.23%) by *T. harzianum*, *R. bataticola* (89.67%) and *S. rolfsii* (86.00%). Maximum seedling vigour index (1866) was observed in seed treatment with *T. viride* @ 4 g/kg. Under field experiment seed treatment with *T. viride* @ 8 g/kg and *P. fluorescens* (10 g/kg) showed 61.79 and 56.75% disease control. Maximum chickpea grain yield (1157 kg/ha) was obtained in the seed treatment with *T. harzianum* @ 4 g/kg as compared to control (728 kg/ha).

Damaram (2012) evaluated five antagonists viz., *T. viride, T. harzianum, T. virens, B. subtilis* and *P. fluorescens* against *Fusarium pallidoroseum*. All the antagonists were found significantly superior in reducing the Fusarium fruit rot over control. Among them *T. harzianum* was found significantly superior in reducing the Fusarium fruit rot severity (10.25 %) followed by *T. virens* (14.25%) on 8th day after both in pre- and post-inoculation treatments. *B. subtillis* found least effective in reducing the rot severity (25.25%).

Basher *et al*. (2014b) reported the seven antagonists, *T. harzianum* showed 87.17% inhibition of *F. solani* at 20% concentration which was followed by *T. viride* (79.49%) and *A. terreus* (76.92%). Similarly, the maximum inhibition of radial growth was observed with *T. harzianum* (86.01%) against *F. oxysporum* which was followed by *A. terreus* (83.33%) and *T. viride* (82.05%) at the same concentration. The inhibition of the test fungi increased with the increase of concentration of culture filtrates in the culture medium.

Ashish kumar and Sahu *(*2015) evaluated the *in-vitro* potentialities of five isolates of *Trichoderma* against phytopathogen *R. solani* by dual culture techniques. A set of six isolates of *R. solani* were also evaluated for their pathogenic aggressiveness and they were found variable in producing the disease on chickpea. The most aggressive isolate produced 75.25% mean disease incidence on chickpea and was further used in confrontation assay with *Trichoderma*. The maximum growth inhibition of *R. solani* (54.2%) was observed by antagonist isolate T_2 (REWA) at 4 days after inoculation.

Al–Ameen *et al.* (2017) reported that dual culture colony interaction, out of four soil fungi, *T. viride* showed the highest growth inhibition on *Colletotrichum musae* (Berk. & Curt.) Arx (58.33%), *Curvularia brachyspora* Boedijn. (61.67%), *Fusarium semitectum* Berk. & Rav. (62.50%), *Fusarium* sp. Link (64.17%) and *Pestalotiopsis disseminat* (Thum) Steyaert (51.54%).

Alka *et al.* (2017*)* revealed that all the antagonists showed varying degree of mycelial growth inhibition of *R. oryzae* over control. Significantly lowest mycelial growth (22.75 mm) with highest growth inhibition was recorded in *T. asperellum* (74.72 %) followed by *T. viride* (27.13 mm) (69.86 %) and *T. harzianum* (29.88 mm) (66.80 %) after 7th day of incubation. While *T. virens* gave lowest mycelial growth inhibition (62.50%).

Cherkupally *et al.* (2017) evaluate the antagonistic effect of seven *Trichoderma* spp., against *F. oxysporum* f. sp*. melongenea* under *in-vitro* conditions. Antagonistic studies for their efficiency, *T. harzianum* showed maximum extent of inhibition 81.1%, followed by *T. koningii 80.0%, T. pseudokoningii* and *T. virens , T. abroviridae* and *T.reesei 77.7%* each by non-volatile compound.

Patra and Biswas (2017) revealed that efficacy of bio-agents and phytoextracts against, *F. oxysporum* f. sp. *ciceri*. Out of which, *T. harzianum* gave maximum inhibition (79.63 %) of mycelia growth of test fungus followed by *T. koningii* with 77.78 % inhibition and least effective is *T. virens* with 55.93 % inhibited fungus growth. In different phytoextracts tested, *Azadirecta indica* showed highest inhibition (16.30 %, 34.56 % and 52.59 %) of test fungus in spite of 2 %, 5% and 10 % respectively, compare to others.

2.4.3: Botanical (Neem):-

Owolade *et al*. (2000) evaluated aqueous extracts of leaves of *Ocium gratissimum, Acalypha ciliata, Vernoxia amygda/ina, Mangifera indica* and *A. indica* for the control of *F. monliforme* on seeds of maize. The seeds were soaked in sterile distilled water containing 10, 20 and 30°/o (W/v) of extracts for 12, 24 and 48 h. All the plant extracts had a significant inhibitory growth on the fungal pathogen. *Acalypha ciliata* was more effective than the other plant extracts.

Bansal and Gupta (2000) tested leaf extracts of seven different plant species viz., *A. indica, Atropa belladonna, Colotropis procera, Ocimum bacillicum, Eucalyptus amygdaline, Ailanthus excelsa* and *Lantana camera* by poisoned food technique at five different concentration viz., 20, 40, 60, 80 and 100 per cent against *F. oxysporum* and reported complete inhibition of mycelial growth and spore germination with 100% leaf extract of *A. indica.*

Rai (2002) evaluated the plant part extract to manage the wilt of pigeonpea caused by *Fusarium udum*. Leaves of *Solanum nigrum,*

Tageonpea erecta, Clerodendron inerme, Lpomea indica, Melia Azaclarachta, Cololropis gigantia, Datura stramonium, A. indica leaves, stem and capsules of *Argemone maxicana,* rhizome of *Zingier officinalis,* neem formulation. All showed their toxical property against *F. udum* inhibiting the growth of organism.

Ali *et al.* (2004) studied the efficacy of neem *(A. indica)* products. Achook, Neemazol and neemgold against sheath blight (caused by *R. solani)* of rice. All the products were significantly effective in reducing the incidence of *R. solani* out of which Neemazol was most effective marketable Neem based formulation.

Singh and Chand (2004) conducted an experiment to determine the efficacy of extracts from *Calotropis procera, D. stramonium, Eucalyptus globulens* (*Eucalyptus globules*), *Jatropha multifida, Azadirachta indica and Allium sativum* on the spore germination of *F. oxysporum* f. sp. *ciceri*. The leaf extract of *A. indica* at 100% completely controlled spore germination, while 100 % *A. sativum* extract only germinated up to 1.7 %.

Mukhtar (2007) determined the antifungal effect of aqueous extracts of four plant species viz., *A. indica, A. Juss., Datura metel L. var. quinquecuspida Torr., O. sanctum* L. and *P. hysterophorus* L. in *in-vitro* study and found that all the plant extracts at 40 % concentration were effective in reducing the mycelial growth of *F. oxysporum* f. sp. *ciceri*. Among these plants extracts, *A. indica* and *D. metel* inhibited fungal growth by 80 % and even at 10 % concentration, both plants extracts had inhibitory effect which had equal potential as fungicides (benomyl 50 WP and carbendazim 50 WP) for the reduction of pathogen growth.

Mossini and Kemmelmeier (2008) observed the efficacy of different concentrations of aqueous neem leaf extract (3.12 to 50 mg/mL) on growth and citrinin production in three isolates of *Penicillium citrinum* was investigated under laboratory conditions. Vegetative growth was assessed, but neem extract failed to inhibit it. Neem leaf extract showed inhibition of toxin production without retardation in fungal mycelia growth.

Mandhare and Suryawanshi (2009) evaluated the antifungal properties of the extracts (@ 10% each) of *A. indica* , *O. sanctum*, *Eucalyptus* sp., *Nerium indicum, A. sativum* and *Zingiber officinale* against *F. oxysporum* f. sp. *ciceri, S. rolfsii*, *R. bataticola* and *A. alternata* by poisoned food technique. The extracts of *A. sativum* and *Z. officinale* completely inhibited the growth of *F. oxysporum* f. sp. *ciceri* whereas *A. indica* extract inhibited the growth of the fungus by 80per cent. The growth of *R. bataticola* was inhibited by 77.7 and 64.4% by the extracts of *A. sativum* and *A. indica*, respectively. The inhibition of the growth of *S. rolfsii* by the extracts of *A. indica* and *A. sativum* reached 100 and 87.5% , respectively. The extracts of *A. sativum, A. indica* and *Z. officinale* inhibited the growth of *A. alternata* by 100, 80.0 and 62.2% respectively.

Adepoju *et al.* (2014) investigate the effects of Neem (*A. indica* A. Juss.) seed oil on four fungi, namely: *Fusarium* sp., *Rhizopus* sp., *Curvularia* sp. and *Aspergillus* sp. which are pathogenic in nature. The extent to which the extract inhibited the growth of the fungi was observed to be different for each of the fungi. Growth inhibition was highest in *Curvularia* sp., while the lowest effect was observed in *Rhizopus* sp

Bashar *et al*. (2014c) reported the all the plant extracts showed varied degree of growth inhibition of the test fungi at different concentrations. Out of the seven plant extracts, *Datura metel* showed 66.67% inhibition of *F. solani* which was followed by *A. racemosus* (65.71%) and *A. indica* (57.14%). The highest inhibition of growth of *F. oxysporum* was observed with *Cassia alata* (74.68%) which was followed by *A. indica* (62.03%) and *A. racemosus* (56.96%).

Brunda Devi *et al.* (2017) reported the *in-vitro* studies to examine the antifungal activity of aqueous leaf extracts of three plants and neem oil against three post-harvest fungal *pathogens viz., Rhizopus arrhizus, S. rolfsii, F. solani*. Among the leaf extracts,

Duranta erecta showed maximum antifungal activity against the pathogens, followed by *Lasonia inermis,* Neem oil*,* and *Cocculus hirsutus.*

The degree of inhibition increased correspondingly with increasing concentrations of the plant extracts. Percentage of inhibitions was high at 20% concentration than 10% concentration, except *S. rolfsii* treated with 10% neem oil. Highest growth inhibitions were recorded in S. rolfsii treated with *D. erecta.*

Rawat *et al.* (2017) investigated the Neem oil to inhibit mycelia growth of *Schizophyllum commune*, *F. oxysporum, F. proliferatum, Coniophora puteana* and *A. alternata* was tested at different concentrations of 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0 and 10%. Results of the study revealed Neem oil concentrations above 2% were significantly inhibitory to all the tested fungi.

MATERIALS AND METHODS

The following material and methods were used in the present investigations entitled **"Studies on seed-borne pathogens of chickpea and their control".** Experiment and related studies were conducted in Department of Plant Pathology, Collage of Agriculture, JNKVV, Tikamgarh (M.P.).

The general laboratory techniques followed in the present study were, those described by Nene and Thapliyal (1993), Dhingra and Sinclair (1995) and Aneja (2003) for preparation of media, sterilization, isolation and maintenance of fungus cultures with slight modification wherever necessary.

The methods and materials used in the present investigation are broadly described under the following heads:

3.1. Glassware, equipments and chemical.
3.2. Sterilization.
3.3. Media preparation.
3.4. Collection of chickpea seed samples.
3.5. Detection and identification of seed pathogens.
3.6. Isolation of predominant seed mycoflora.
3.7. Location of fungi in seed.
3.8 *In-vitro* study of the antagonistic effect of certain fungicides, botanicals and fungal and bacterial antagonists against predominant seed mycoflora.
3.9. Statistical analysis.

3.1. GLASSWARE, EQUIPMENTS AND CHEMICALS.

3.1.1 Glassware:

Standard "Borosil" make glass wares likes Petri dishes, beakers, funnels, pipettes, conical flasks, culture tubes, measuring cylinders etc. were used during the course study. Initially all glassware were thoroughly washed with detergent and rinsed in running tap water. Subsequently they

were kept in cleaning solution for 24 h and finally rinsed in distilled water for 3 to 4 times and air dried.

Composition of cleaning solution

Potassium dichromate ($K_2Cr_2O_7$) : 60 g

Concentrated sulphuric acid (H_2SO_4) : 60 ml

Distilled water : 1000 ml

3.1.2 Equipments

Compound microscope (10x, 40x, magnification) was used for observing the fungi. Chemicals were weighed on a single pan electronic balance with a sensitivity of 0.001 g. Hot air oven and autoclave were used for sterilization of glassware and media respectively. The test materials were incubated in BOD incubator at different temperatures and the cultures were stored in the refrigerator.

3.1.3 Chemicals

Chemicals of Analytical Reagent (AR) and Guaranteed Reagent (GR) grades of standard make were used. The pH of the media was adjusted using either 0.1 N HCl or 0.1 N NaOH.

3.2 STERILIZATION.

Glassware *viz.,* Petri plates and test tubes were wrapped with brown paper and sterilized in hot air oven at 160℃ for 90 minutes. Inoculation loop, cork borer and scalpel were sterilized by dipping in alcohol and heating over flame till red hot.

Different media and water used in this experiment were sterilized by autoclaving as mentioned in media preparation. Work benches and surface of laminar air flow chamber were sterilized by wiping with cotton dipped in ethyl alcohol solution.

Sodium hypochlorite (NaOCl) 1% solution was used to surface sterilizes the seeds for 2 min followed by three washings with sterile distilled water and rectified spirit for hands.

3.3 MEDIA PREPARATION

Potato Dextrose Agar (PDA) was used to isolate and maintain the cultures throughout the investigation period. Nutrient agar (NA) medium was used for culturing and maintenance of bacterial antagonists *viz*., *Pseudomonas* spp.

3.3.1 Potato Dextrose Agar (PDA)

Potato : 200 g
Dextrose : 20 g
Agar-agar : 20 g
Distilled water : 1000 ml
pH : 6.5

200 g of peeled potato pieces were boiled in 500 ml of distilled water in a 1000 ml beaker till the pieces get softened. The extract was filtered through a double layered muslin cloth into which 20 g of dextrose was added. To another 500 ml of distilled water in another 1000 ml beaker, 20 g of agar was added and melted till it gets dissolved. Both the solutions were mixed in another 1000 ml beaker. The final volume of the medium was made up to 1000 ml by addition of sterile distilled water. The pH of the medium was adjusted to 6.5 with 0.1 N NaOH or 0.1 N HCl as the case may be with the pH meter. The medium was dispensed to test tubes and conical flasks of 8.0 ml and 100 ml capacity respectively. The medium was sterilized in an autoclave at 15 psi for 15 min.

3.3.2 Nutrient Agar (NA) Medium

Nutrient agar medium was used for culturing and maintenance of bacterial antagonists *Pseudomonas* spp.

Twenty eight grams nutrient agar medium (Hi-media make) was dissolved in1000 ml of distilled water in one liter conical flask and the medium was sterilized in an autoclave at 15 psi for 15 minutes.

3.4. COLLECTION OF CHICKPEA SEED SAMPLES.

Untreated chickpea seed samples were collected from different chickpea growing areas of district Tikamgarh *viz*. block- Tikamgarh, Baldevgarh, Prithvipur, Palera, Jatara and Nivari at harvesting stages during *rabi* 2017-18. The location of the survey was conducted on 5-field of two village of each block of Tikamgarh district. The sample was collected at randomly from chickpea field. 10-samples are collected from each field during surveying. Following is the details of the collected seed samples.

Table-1: Survey and collection of seed samples from chickpea growing area of district Tikamgarh (M.P.).:

Blocks	**Village (1)**	**Village (2)**
Tikamgarh	Ganeshganj	Kundeshwar
Baldevgarh	Bhiloni	Lodwani Khas
Prithvipur	Mohanpura	Sujanpura Jagir
Palera	Jewar	Mannpura
Jatara	Kurrai	Vijaypura
Nivari	Tila	Pipra

*The collected seed samples were shade dried and stored in paper bags at ambient storage temperature of 28 ± 2 ℃ for further usage.

3.5. DETECTION AND IDENTIFICATION OF SEED PATHOGENS.

All the seed samples were examined by visual seed inspection and occurrence of seed mycoflora was analyzed. For detection and isolation of seed mycoflora associated with chickpea seeds, the four detection

methods *viz*., dry seed inspection, seed washing method, Standard blotter method and Agar plate method.

3.5.1 Dry seed inspection (ISTA, 2012).

It is a very simple and preliminary method for testing the seed health. The dry seed samples were examined for impurities such as:

a. Inert matter: in includes plant debris, spotted, unfilled and chaffy grains, insect *etc.* It should also be incubated either on blotter or, agar media and examined, after a standard period of infection.

b. Symptoms: such as discoloration staining and similar indications of infections, including resting hyphae on the seed surface, spores on the seed as well as mechanical damage.

Seed samples were examined first by naked eyes and the observed under the stereo binocular microscope for confirmation of the above impurities in proper way.

3.5.2 Seed Washing Method (ISTA, 2012):

The washing test is a seed health testing method, employed to test seeds for seed-borne pathogens, the inoculums of which is present loosely on the seed surface. 2 g of chick pea seed samples were taken in a test tube with 10 ml of sterilized water and shaken for 10 minutes on a mechanical shaker. The suspended spores were concentrated by centrifugation at 3000 rpm for 20 minutes. The supernatant was discarded and the spores are again suspended in 2 ml of lacto phenol and the suspension was then examined under the microscope for the presence of fungal spores.

3.5.3. Standard Blotter Method (Doyer, 1938):

400 seeds of each sample were tested by employing standard blotter method in 3 replications. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized Petri plates after draining excess water. Untreated seeds were placed at the rate of 25 seeds per Petri plate at equal distance. The plates were incubated at room temperature (20 ± 2º C) under alternate cycles of 12

hours NUV light and darkness. After eight day of incubation the seeds were examined under stereoscopic – binocular microscope for the associated fungi and they were identified based on habit and colony characters.

3.5.4. Agar plate method with potato dextrose agar (Muskett and Malone, 1941):

400 seeds of moderately infected each Chickpea sample were surface sterilized with 1 per cent sodium hypochlorite solution for 1-2 min and then placed at the rate of 10 seeds per Petri plate containing 20 ml of potato dextrose agar. The Petri plates were incubated for 7 days as described under standard blotter method. After 7 days of incubation the fungal growth was examined under stereoscopic binocular microscope.

3.5.5 Identification of fungi

Identification of various fungal cultures pertaining to chickpea seed pathogens was done using the key given by Barnett and Hunter (2003), Booth (1971) and Subramanian (1971). The identification of fungi was done based on the spore morphology and colony character (Ram Nath *et al*, 1970). The identified suspension was *R. bataticola* bearing the spore mycelium, *Rhizopus* spp. bearing the sporangiospore, *Aspergillus niger* bearing the conidiophores, *Penicillium* spp. bearing the conidiophores and *F*. *oxysporium* f. sp. *ciceri* bearing the mycelium.

3.6 ISOLATION OF PREDOMINANT SEED MYCOFLORA

Fungal pathogen detected from standard blotter method was adopted for isolation of seed pathogens. The fungal hyphae were picked up from surface of the seed and transferred onto Petri plate containing PDA media. The culture was further purified following single spore isolation technique (Tuite, 1969). Thus obtained pure culture was maintained on PDA slants. Such culture tubes were preserved in a refrigerator at 4oC and renewed once in a month for further studies.

3.7 LOCATION OF THE FUNGI IN SEED

The location of fungi in seed was studied by employing component plating technique (Maden *et al*, 1975). Naturally infected chickpea seed samples were used for the study. 10 seeds were washed four times with tap water then surface sterilized in one per cent sodium hypochlorite solution for two minutes. These seeds were again washed with sterile water and soaked in water for 2 hrs and then the seeds were dissected aseptically using sterile needle and forceps. The separated seed parts *viz*., seed coat, cotyledon and radicle were plated immediately before drying on potato dextrose agar plates. The plates were incubated at 20 ± 2ºC for seven days, the seed component were examined under stereoscopic binocular microscope for the presence of fungal structures in different seed parts.

3.8. *IN-VITRO* STUDY OF THE ANTAGONISTIC EFFECT OF CERTAIN FUNGICIDES, BOTANICALS AND FUNGAL AND BACTERIAL ANTAGONISTS AGAINST PREDOMINANT SEED MYCOFLORA.

3.8.1 Evaluation of seed dressing fungicides

This study was carried out to know the efficacy of different seed dressing fungicides in eliminating the seed-borne fungal infections in the infected seed sample. The fungicides were tested initially under *in vitro* condition by using poison food technique (Schmitz, 1930).

The trade name, common name and chemical names of fungicides used in the experiment are given below.

Table- 2: **List of fungicides used in the experiment.**

S.No.	Common name	Trade name	Chemical name
1.	Carbendazim (0.2%)	Bavistin 50% WP	2-(methoxy carbomyl)-benzimidazol
2.	Carboxin (0.2%)	Vitavax powder 75% WP	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide
3.	Mancozeb (0.2%)	Maneb 75%WP	Ethylene-bisdithiocarbamates
4.	Carbandazim (0.1%) + Carboxin (0.1%)	Bavistin + vitavax powder	2-(methoxy carbomyl)-benzimidazol + 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide

All the fungicides were tested at recommended by adopting poisoned food technique. The test pathogen was grown on PDA medium in Petri plates for seven days prior to setting up of experiment. The required fungicidal suspension was added to the melted PDA medium to obtain the desired concentration on the basis of active ingredients present in the chemical.

20 ml of poisoned medium was poured in each Petri plate. Suitable checks were maintained without addition of fungicides. A mycelial disc of five mm diameter was taken from the periphery of 7 days old colony and placed in the centre and incubated at 28 ± 2℃ for full growth of the fungus. Three replications were maintained for each treatment. The radial growth of the colony was measured in two directions and average was recorded. Per cent inhibition was recorded by using the formula given by Vincent (1947) as under:

$$PI = [(C–T)/C] \times 100$$

Where,

PI = Per cent inhibition

C = Growth in control

T = Growth in treatment

3.8.2 Evaluation of plant extract:

These plant extracts were tested initially under *in-vitro* condition by using poison food technique. The fresh leaves were grounded in a blender with distilled water. The extract was filtered through double layered muslin cloth. The extracts were tried at concentration of 5 per cent for seed treatment, prepared by diluting the extract in distilled water.

Table-3: Name of the bio-pesticide used in experiment

Botanical name	Common name	Family	Used plant parts
A. indica	Neem	Meliaceae	Leaf

Preparation of cold aqueous extract

Fresh plant material were collected and washed first in tap water and then in distilled water. 100 grams of fresh sample was chopped and then crussed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth and the filtered was used as stock solution. To study the antifungal mechanism of plant extract, the poisoned food technique was used (Schmitz, 1930). 5 and 10 ml of stock solution was mixed with 95 ml and 90 ml of sterilized molten PDA media respectively so as to get 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. 20 ml of medium was poured into sterile Petri dishes. Mycelium of 5 mm size disc from periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed on the centre of each agar plate. Control was also maintained by growing the pathogen on PDA plates. Each treatment was replicated thrice and plates were incubated at 20 ± 2ºC till control plates reached the radial growth of 90 mm. The per cent inhibition over control was calculated according to the formula given by Vincent (1947).

3.8.3 *In-vitro* evaluation of bio-agent.

The micro-organisms like *T. viride, Pseudomonas* spp. were evaluated for their antagonistic effect under *in-vitro* condition against isolated seed borne pathogens by dual culture technique.

a. Dual culture technique

In dual culture, 20 ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. Fungal antagonist was evaluated by inoculating the pathogen at one side of Petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this actively growing cultures were used. In case of evaluation of bacterial antagonist two mycelial discs of pathogens were inoculated and bacterial antagonist was streaked in the centre of the plate. Each treatment was replicated four times. After required period of incubation i.e., after control plates reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to formula given by Vincent (1947).

3.9. STATISTICAL ANALYSIS

The experimental design Complete Randomized Block Design (CRBD) at 1 to 5% level of significance was used for statistical analysis.

EXPERIMENTAL FINDINGS

The results of the present investigation on “Studies on seed- borne pathogens of chickpea and their control.” The experiment was conducted in laboratory at Department of Plant Pathology, College of Agriculture, Tikamgarh (M.P.) during 2017-18 and results were presented in this chapter under following heads.

4.1. SURVEY AND COLLECTION.

Studies on the extent of seed borne pathogens of chickpea in Tikamgarh district of Madhya Pradesh had not conducted earlier, so for the information on extent of seed borne pathogen of Tikamgarh district are not available. A survey was conducted in this district during *Rabi* season 2017-18 for collection of seed samples from different chickpea growing areas of Tikamgarh district. The seed samples were collected from 6-blocks *viz*., Tikamgarh, Baldevgarh, Nivari, Jatara, Prithivipur and Palera. In each block selected two village *viz.,* Ganeshganj and Kundeshwar (Tikamgarh), Bhiloni and Lodhwani Khas (Baldevgarh), Mohanpura and Sujanpura Jagir (Prithivipur), Tewar and Mannpura (Palera), Kurrai and Vijaypura (Jatara) and Tila and Pipra (Nivari) and in two village five chickpea fields were randomly selected at crop maturity stage. The collected samples were analyzed at laboratory for extent of seed borne pathogens by using seed health testing methods as mentioned in Method and Materials (Table-1).

4.2. MORPHOLOGICAL CHARACTERISTICS.

In the present investigation, five fungal species *viz.*, *R. bataticola, F. oxisporum* f. sp*. ciceri*, *A. niger, Rhizopus* sp., and *Penicillium* sp. were recorded on chickpea seed.

The predominant fungal species observed on chickpea seed was identified as all isolated fungus and pure culture was maintained on PDA by periodical transfers. The colony character and spore morphology of the mycoflora observed were given below:

4.2.1. *Rhizoctonia bataticola*

Asexual fruit bodies and spores lacking, sclerotia brown or black, variable in form, frequently small and loosely formed, formed among and connected by mycelia threads; hyphae of mycelium brown, with long cells, septa of branch set off from main hypha; parasitic, chiefly on roots and other underground parts of plants.

4.2.2. *Fusarium oxysporum*

F. oxysporum f. sp. *ciceri* produced white to light orange, aerial mycelium and sporodochia on incubated seed. Mycelium profusely branched, covering the entire seed and white to light pink. Sporodochia rarely produced, but if present completely covered by aerial mycelium. Micro-conidia abundant and produced on short unbranched monophialides in small dry false heads, hyaline single celled, oval to cylindrical, straight to slightly curved and 2.5-3.5 x 5-11 µm. Macro-conidia sparse and produced on branched macroconidiophores, fusoid with pointed ends, hyaline, with 3-5 septa, measuring 3.5-4.5 x 25-65µm. Chlamydospores usually intercalary and produced singly or in pairs, globose to sub-globose thick walled and smooth or rough surfaced.

4.2.3. *Aspergillus niger*

Colony consist of a compact to fairly loose, white to fainty yellow basal mycelium, which bears abundant, erect and usually crowded conidial structures. Conidial heads are typically large and black, compact at first, spherical or split into two or looser to reasonably well defined columns. Conidiophores are smooth, hyaline or fainty brownish near the apex. Two series of conidia bearing cells (supporting cells and phialides) produced. Conidia typically spherical at maturity.

4.2.4. *Rhizopus* sp.

Very fast growing colony, white cottony initially becoming brownish grey to blackish-grey depending on the amount of sporulation. Sporangiophores smooth walled, non - septate, simple or branched,

Plate – 1: Survey done during harvest stage in block - Tikamgarh (Ganeshganj)

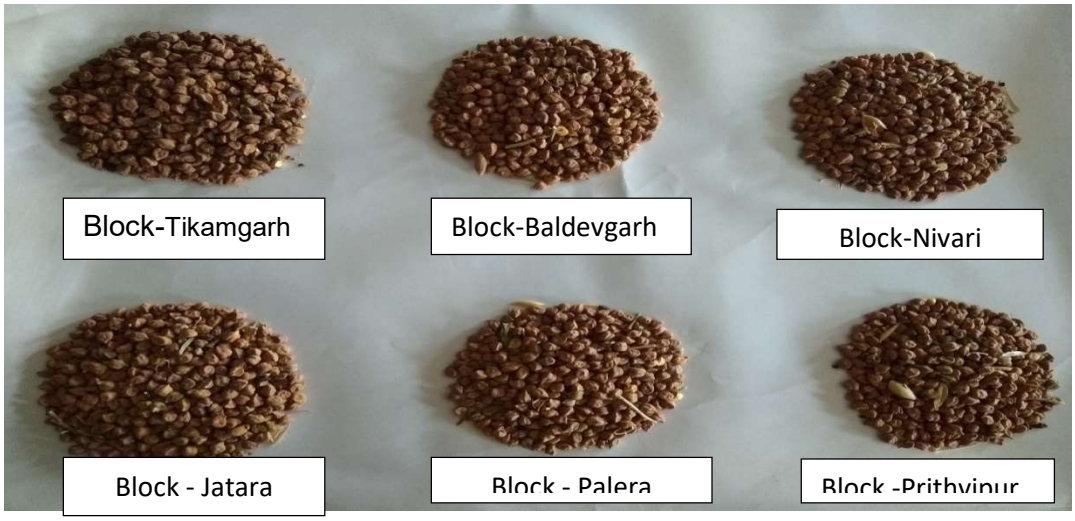


Plate -2: Collection of seed samples from different chickpea growing area of Tikamgarh block

arising from stolons opposite rhizoids usually in groups of 3 or more. Sporangia globose, often with a flattened base, grayish black, powdery in appearance and many spored.

4.2.5. *Penicillium* sp.

Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in phialides; conidia (phialospores) hyline or brightly coloured in mass, 1-celled, mostly globose or ovoid, in dry basipetal chains**.**

4.3. DETECTION OF SEED-BORNE MYCOFLORA

Chickpea seeds collected from major growing areas of Tikamgarh district (M.P.) during *rabi* season in 2017-18, were subjected to most commonly used seed health tests methods *viz.,* dry seed inspection, seed washing test, standard blotter paper and agar plate method to detect seed-borne pathogens which was associated with different chickpea seeds samples and the results were obtained are presented here under following heads.

4.3.1. Dry seed inspection method (ISTA, 2012)

The observations on the seeds morphological characterizes were recorded by the help of naked eyes and hand lenses by using the dry seed inspection method. The discoloration of seeds were observed in samples collected from the block Baldevgarh, Jatara and Palera while None of seed samples were found discolorated of Tikamgarh, Nivari and Prathavipur. The spotted symptoms appeared on seeds only of Baldevgarh and Nivari blocks. All blocks seeds were found mechanically damaged except Baldevgarh. Chaffy type's seeds weight was not found in Nivari blocks but all samples from different blocks were found unfilled (Table-4)

The maximum poor morphologically appearance *viz*., discolorated, spotted and unfilled or chaffe grain was recorded in Baldevgarh, Nivari and Palera in compared to others blocks seed samples.

Table- 4: Morphological observations of collected seed samples from different chickpea growing area of district Tikamgarh (M.P.)

Block	Morphological characteristics of seed samples			
	Discoloration	Spotted	Mechanically damage	Unfilled or chaffy
Tikamgarh	-	-	+	+
Baldevgarh	+	+	-	+
Jatara	+	-	+	+
Nivari	-	+	+	-
Prithvipur	-	-	+	+
Palera	+	-	+	+

* Where present (+) and absence (-).

4.3.2. Seed washing method

The spore morphology of the fungus present outside the seeds which was identified through seed washing test method (Table 5). The seed samples containing the fungus mainly *Rhizoctonia bataticola, Rhizopus* spp*., Aspergillus Niger, Penicillium* spp*. and Fusarium oxysporum* f. sp. *ciceri.*

The suspension which was identified under the microscope contents the spore morphology of mycelium in *R. bataticola and F. oxysporum* f. sp. *ciceri,* conidiophores in *A. niger and Penicillium* spp. and sporangiophore in *Rhizopus* spp.

Among the seed borne pathogens in the *Rhizoctonia bataticola* is present in the block of Tikamgarh, Prithvipur and Palera. *Rhizopus* spp. which was present in Jatara and Prithvipur. *Aspergillus niger* was present in all the block except Prithvipur, *Penicillium* spp. which was observed in Baldevgarh, Jatara, Nivari and Prithvipur. *F. oxysporum* f. sp. *Ciceri* which was absent in all blocks in Tikamgarh district.

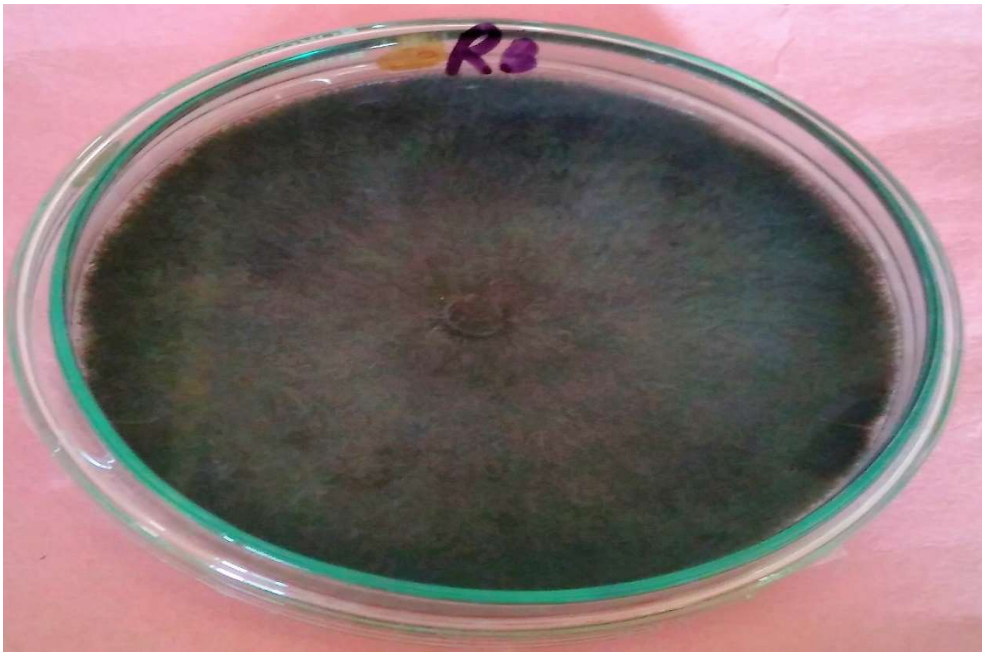


Plate- 3: Pure culture of *Rhizoctonia bataticola.*

Plate- 4 – Pure culture of *Fusarium oxysporum* f.sp. *ciceri.*

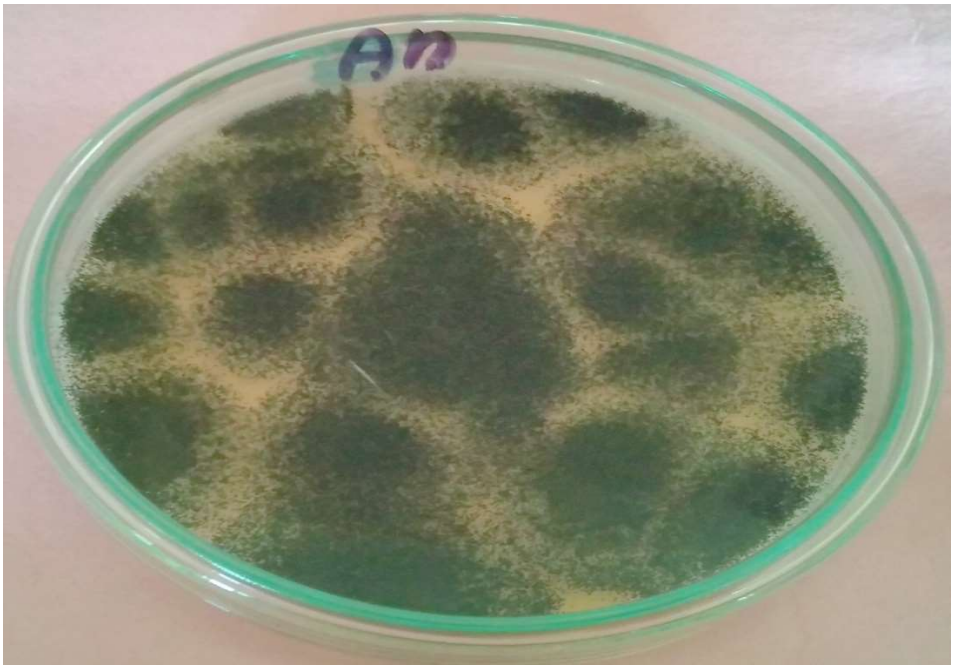


Plate – 5: Pure culture of *Aspergillus niger.*

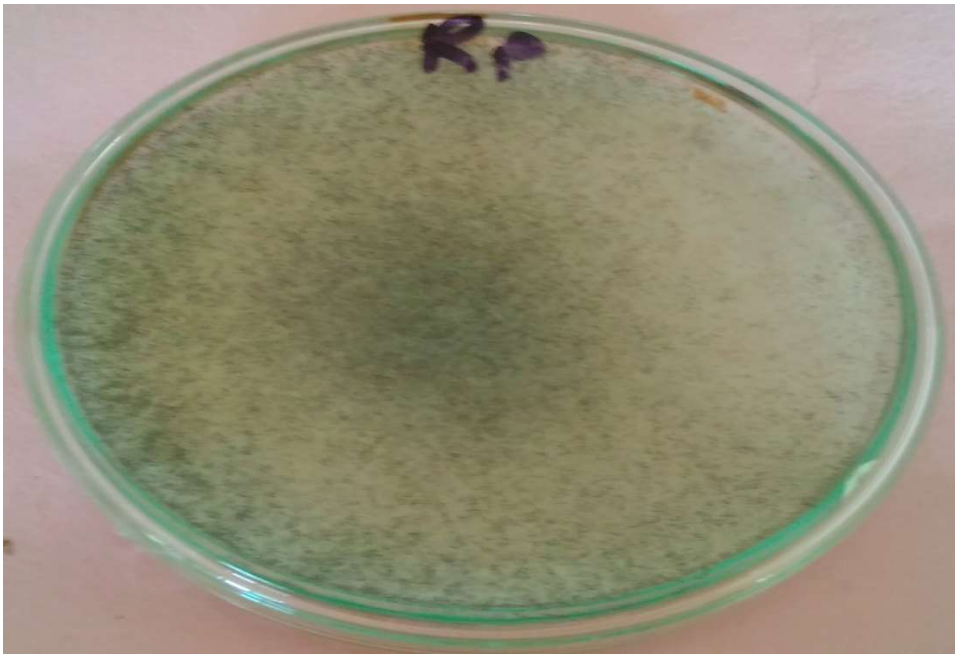


Plate- 6: Pure culture of *Rhizopus* spp.

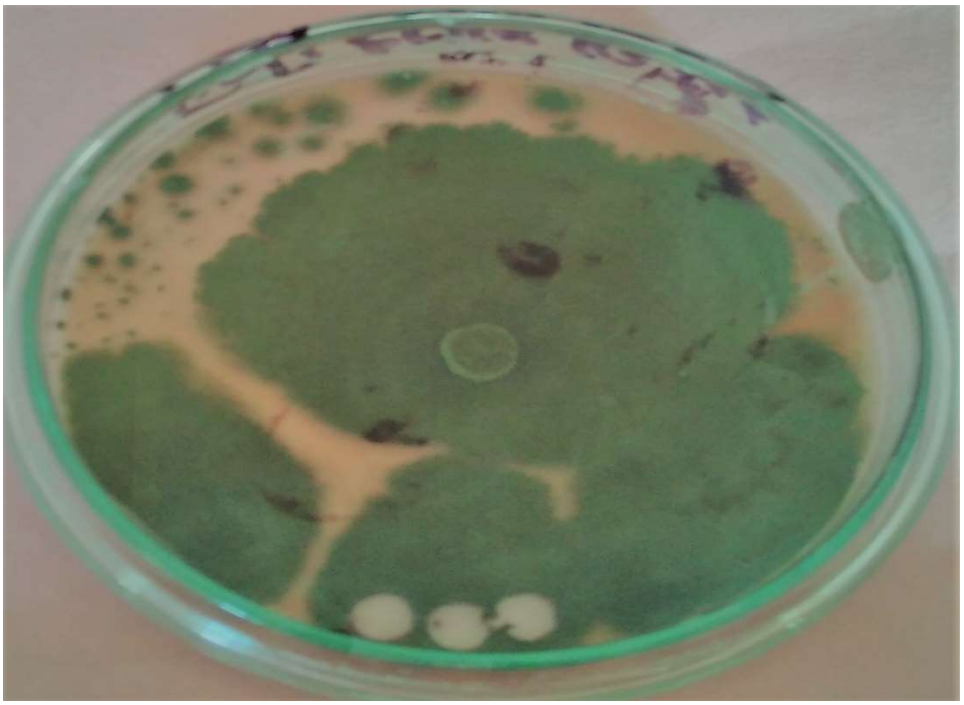


Plate – 7: Pure culture of *Penicillium* spp.

Plate 8. Healthy and infected seeds of chickpea showing damage caused by seed-

Table-5: Observation of spore morphology of the fungus present outside the seeds

Block	*R. bataticola*	*Rhizopus* spp	*A. nigar*	*Penicillium* spp.	*F.oxysporum* f. sp. *ciceri*
Tikamgarh	+	-	+	-	-
Baldevgarh	-	-	+	+	-
Jatara	-	+	+	+	-
Nivari	-	-	+	+	-
Prithvipur	+	+	-	+	-
Palera	+	-	+	-	-

* Where present (+) and absence (-) of the seed-borne pathogens.

4.3.3. Standard blotter paper method.

The observations of the seeds are done by the standard blotter paper method. The observations are recorded as number of colonies (Table-6).

Among all seed borne pathogens the highest average number of colonies was recorded for *F. oxysporum* f. sp. *ciceri* (05), followed by *A. nigar* (4.33), *R. bataticola* (2.83), *Rhizopus* spp. (2.16) and *Penicillium* spp. (1.16).

Among the block, the highest number of colonies were recorded maximum in Tikamgarh block (3.6) followed by Nivari (3.4), Baldevgarh (3.2), Prithvipur (03), Palera (2.8) and Jatara (2.6).

Table- 6: The number of colonies present in the different seed samples detected to standard blotter paper method (Doyer, 1938).

Block	*R. bataticola*	*Rhizopus* spp.	*A. nigar*	*Penicillium* spp.	*F. oxysporum* f. sp.*ciceri*	Average
Tikamgarh	02	00	05	03	08	**3.6**
Baldevgarh	00	03	08	00	05	**3.2**
Jatara	04	00	04	02	03	**2.6**
Nivari	03	04	04	00	06	**3.4**
Prithvipur	03	06	02	00	04	**3.0**
Palera	05	00	03	02	04	**2.8**
Average	**2.83**	**2.16**	**4.33**	**1.16**	**5.0**	

4.3.4. Agar plate method with potato dextrose agar.

The observations of the seeds are done by the Agar plate method with potato dextrose agar. The observations are recorded as number of colonies present in the Tikamgarh district.

Among all seed borne pathogens the highest average of number of colonies was recorded for *A. nigar* (04), followed by *F. oxysporum* f. sp. *ciceri* (2.66), *R. bataticola* (2.16), *Rhizopus* spp. (1.5) and *Penicillium* spp. (1.33).

Among the block the highest number of colonies were recorded maximum in block Tikamgarh (03) followed by Baldevgarh (2.6), Prithvipur (2.4), Palera (2.2) Nivari (02), and Jatara (1.8) (Table- 7).

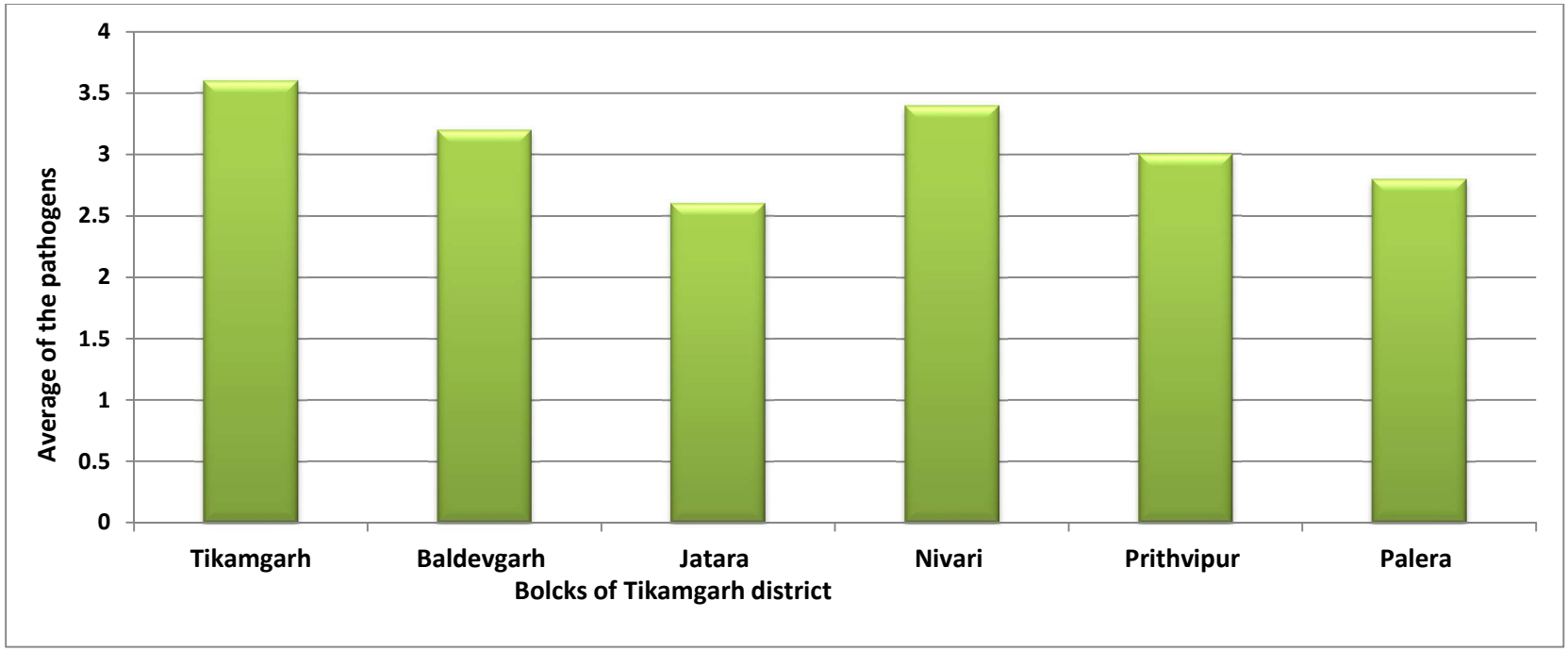


Figure –1: Average number of colonies present in the different seed samples detected to standard blotter paper method.

Table- 7: The number of colonies present in the different seed samples.

Block	Number of colonies of different seed-borne pathogens					
	R. bataticola	***Rhizopus* spp.**	***A. nigar***	***Penicilliu m* spp.**	***F.oxysporum* f.sp.*ciceri***	**Average**
Tikamgarh	03	03	06	00	03	**3.0**
Baldevgarh	03	00	05	02	03	**2.6**
Jatara	00	04	03	02	00	**1.8**
Nivari	00	00	05	01	04	**2.0**
Prithvipur	04	02	02	00	04	**2.4**
Palera	03	00	03	03	02	**2.2**
Average	**2.16**	**1.5**	**4.0**	**1.33**	**2.66**	

4.3.5 Location of the fungi in seed.

The location of fungi in the seeds was studied by employing component plating technique, presented in Table 8. Naturally infected chickpea seed sample which were collected from different locations of district Tikamgarh was used for this study. The presence of seed-borne pathogen in the separated seed parts *viz*., seed coat, cotyledons and radicle were recorded as. The colonies of *F. oxysporum* f. sp. *ciceri* was noticed on the all the component of the seed that were seed coat, cotyledon and radicle, where as colonies of *A. niger* was noticed only on seed coat and cotyledon, but not on the radicle.

Among the component of the seeds maximum number of colonies were observed in seed coat (03) followed by cotyledon (02) and radicle (02) of *F. oxysporum.* f.sp. *ciceri*. Regarding *A. niger* the highest number of colonies were noticed in seed coat (02) followed by cotyledon (01) and completely absence in radicle

Table – 8: The number of seed borne pathogens colonies on different seed components in PDA by using seed plating component techniques

Components of seed	No. of pathogens colonies on the PDA		
	F. Oxysporum	***A. niger***	**Average**
Seed coat	03	02	**2.5**
Cotyledon	02	01	**1.5**
Radicle	02	00	**01**
Average	**2.3**	**01**	**2.5**

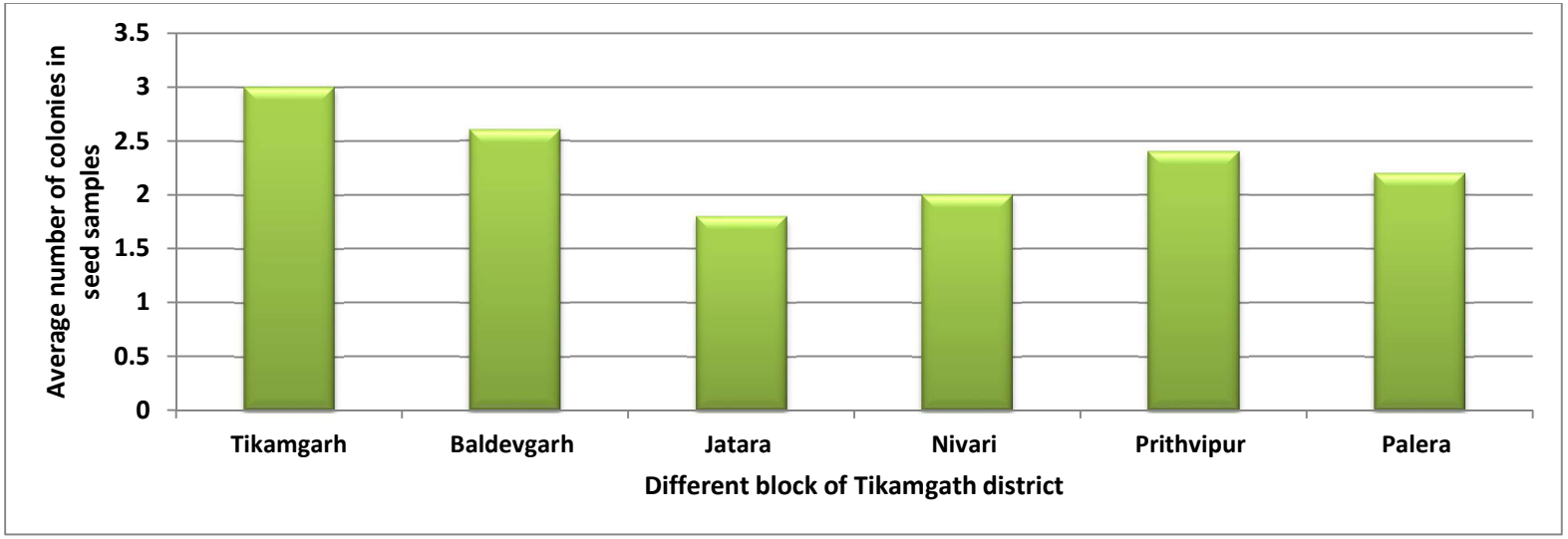
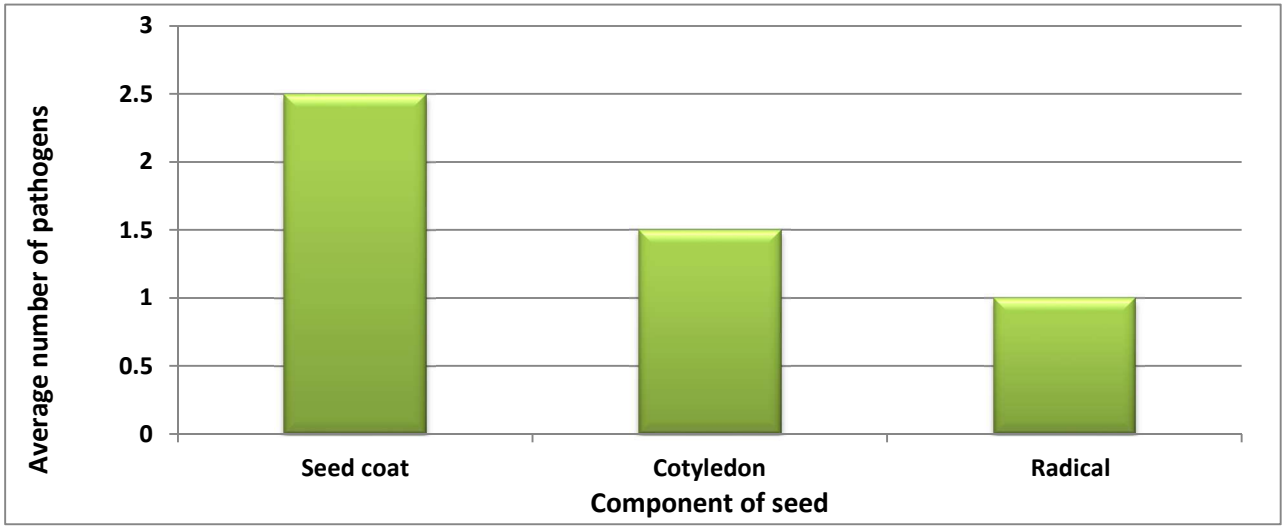


Figure -2: Average number of colonies present in the different seed samples detected to Agar Plate method.



Figure– 3: Average number of seed borne pathogens colonies on different seed components in PDA by using seed plating

4.4 EVALUATION OF FUNGICIDE, BIO-AGENTS AND BIO-PESTICIDE AGAINST MAJOR SEED BORNE PATHOGENS OF SEED *IN-VITRO.*

4.4.1 Evaluation of Fungicides and Bio-pesticide.

Efficacy of four fungicides Carbendazim (0.2%), Carboxin (0.2%), Mancozeb (0.2%) and Carbendazim (0.1%) + Carboxin (0.1%) were evaluated against all seed borne pathogens adopting poisoned food technique. Observations on the radial growth of the all seed borne pathogens were recorded after 7 days of incubation. The percent inhibition of the all pathogens over control was calculated and presented in Table-9.

Among the different fungicides and bio-pesticide used in the *in-vitro* condition adopted by Poisoned Food technique. The superiority in controlling the inhibition of seed borne pathogens was managed by Carbendazim + Carboxin followed by Carboxin, Carbendazim and Mancozeb. Whereas the Neem extract (5%) was inferior in controlling the inhibition of the radial growth of the seed-borne pathogens.

Regarding the seed-borne pathogens the growth of the *R. bataticola* was effectively inhibited the by all the fungicides (100%) except Neem extract (23.28%). Carbendazim, Carboxin and Carbendazim + Carboxin, Mancozeb inhibited maximum growth (100%) of the pathogens over Neem extracts (23.08%).

Figure– 3: Average number of seed borne pathogens colonies on different seed components in PDA by using seed plating

Plate- 9: Seed-borne pathogens identified through Standard blotter paper method.

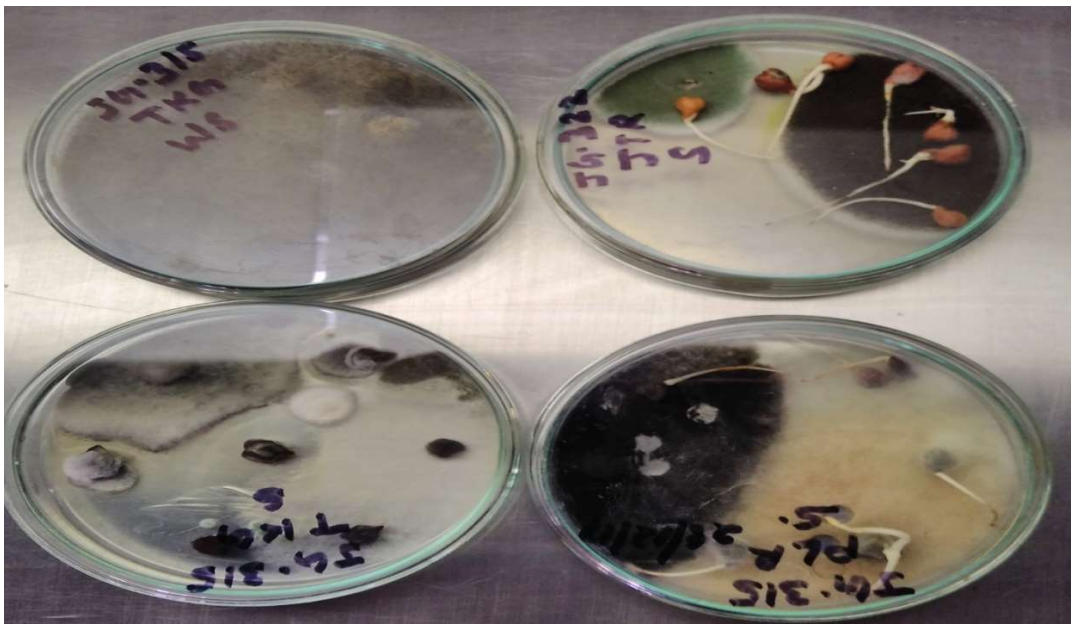


Plate- 10: Number of colonies identified through Agar plate method.

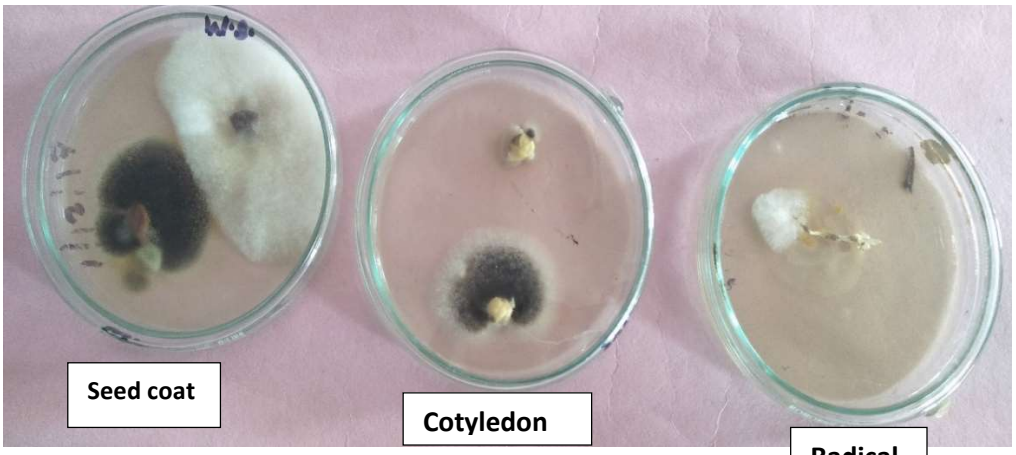


Plate – 11: Number of colonies present in different component of seeds through Employing component plating technique.

Similar trend was observed in *F. oxysporum* f. sp. *Ciceri, Penicillium* spp. and *A. niger.* The maximum growth was inhibited by fungicides Carbendazim, Carboxin and Carbendazim + Carboxin (100%) followed by Mancozeb (77.49, 75.60 and 74.52%). Whereas Neem extract was resulted in poor inhibition of the pathogens (74.56, 48.41 and 43.97%).

The seed-borne pathogens *Rhizopus* spp. was effectively only inhibited by the Carbendazim + Carboxin (100%) followed by Carboxin (42.34%), Mancozeb (30.98%), Carbendazim (23.49%) and the lowest inhibition of the pathogens radial growth was showed by the Neem extract (23.43%).

Table–9: Percent inhibition of radial growth of seed-borne pathogens of chickpea in *in-vitro*.

Fungicides and Bio-pesticide	Mean growth (mm) of seed-borne pathogens and inhibition over control (%).									
	R. bataticola		***A. niger***		***F. oxysporum* f. sp. *Ciceri***		***Penicillium* spp.**		***Rhizopus* spp.**	
	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)
Carbendazim (0.2%)	00.00	100	00.00	100	00.00	100	00.00	100	33.00	23.43
Carboxin (0.2%)	00.00	100	00.00	100	00.00	100	00.00	100	24.86	42.34
Mancozeb (0.2%)	00.00	100	09.86	74.52	08.73	77.49	09.60	75.60	29.76	30.98
Carbendazim (0.1%)+ Carboxin(0.1%)	00.00	100	00.00	100	00.00	100	00.00	100	00.00	100
Neem extract (5%)	33.16	23.28	21.96	43.97	9.86	74.56	19.17	48.41	33.06	23.33
Control	43.23	-	39.20	-	38.80	-	37.16	-	43.13	-
C.D at 5 %		**0.46**		**0.72**		**0.55**		**0.86**		**0.92**
S.E.(m)±		**0.14**		**0.23**		**0.17**		**0.27**		**0.29**

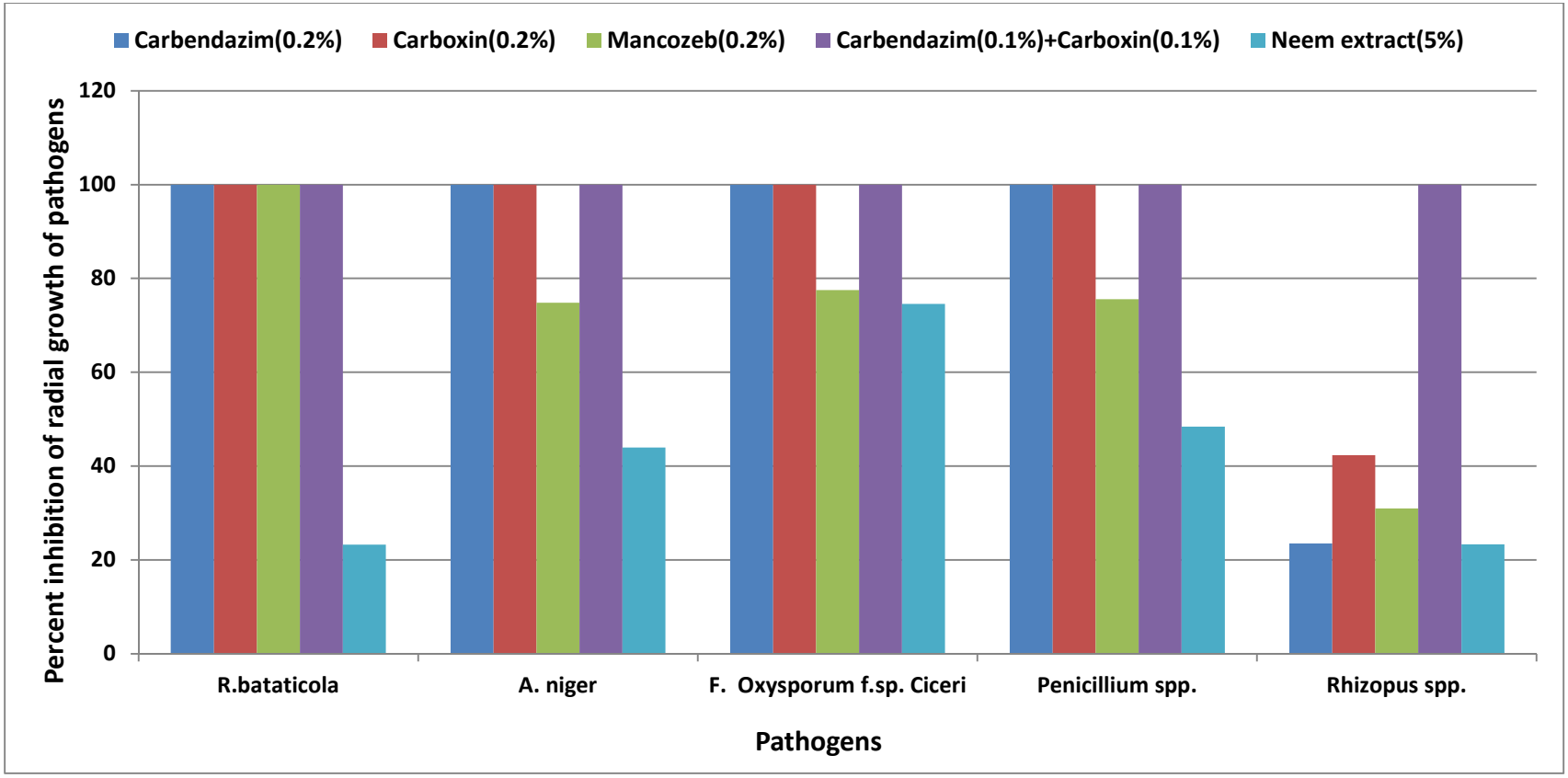
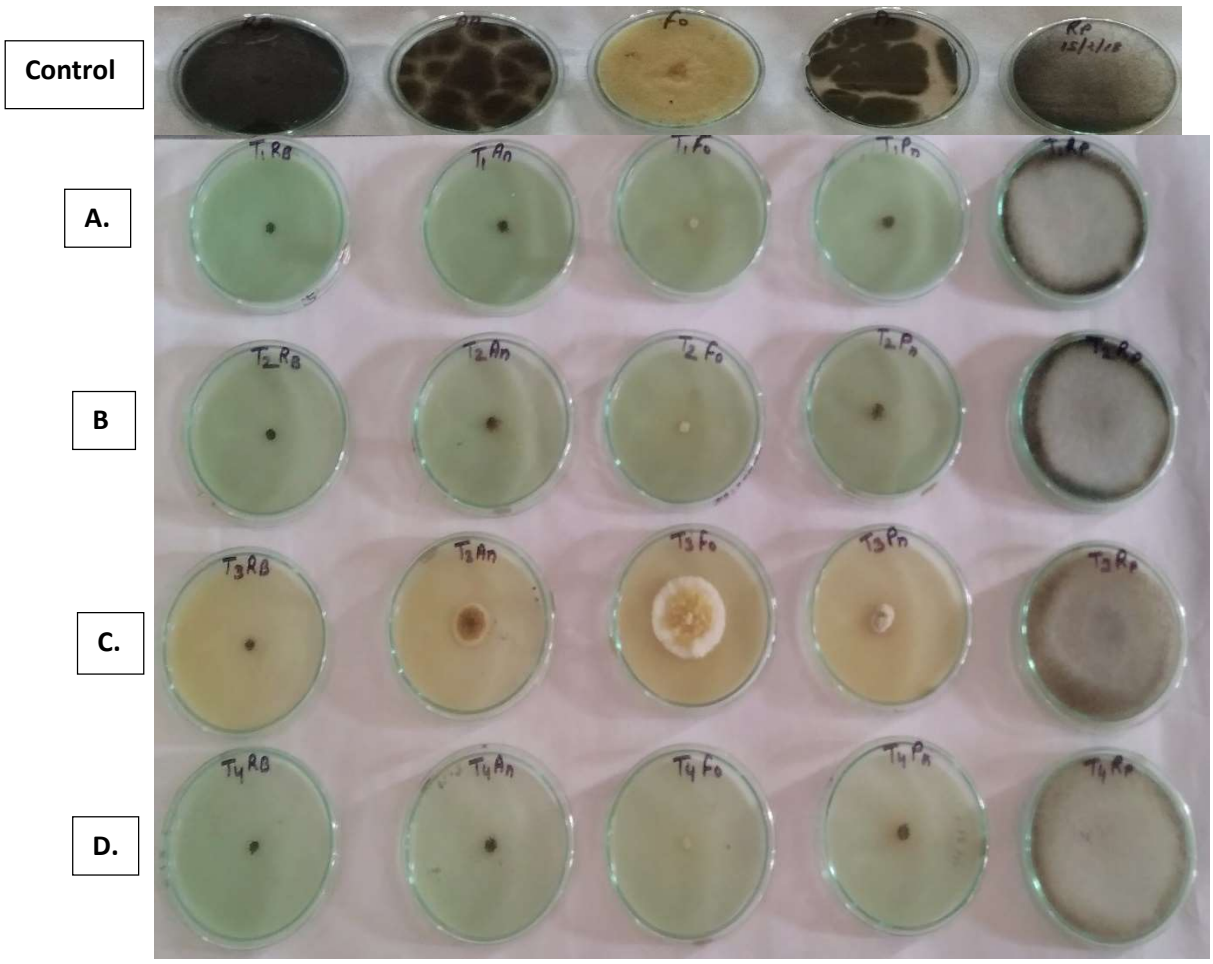


Figure-4: - Percent inhibition of seed-borne pathogens of chickpea over control by Poisoned Food technique.



A. Carbendazim 0.2%, B. Carboxin 0.2%, C. Mancozeb 0.2%
D. Carbendazim0.1%+Carboxin 0.1%.

Plate- 12: Evaluation of fungicide and bio-pesticide against major seed-borne pathogens.

4.4.2. *In-vitro* evaluation of bio-agents (*T. viride* and *Pseudomonas* spp.) by dual culture technique.

The antagonistic micro-organism *viz., T. viride* and *Pseudomonas* spp. were evaluated against test pathogens *viz., R. bataticola, Rhizopus* spp., *A. niger, Penicillium* spp., *F. oxysporum* f. sp. *ciceri.* by Dual Culture technique and percent inhibition was recorded based on radial growth of the test pathogens (Table-10). Significant inhibition of the radial growth was recorded.

The *T. viride* showed the highest growth inhibition on the pathogens *F. oxysporum* f. sp. *ciceri* (50.35%) followed by *R. bataticola* (41.87%), *Penicillium* spp. (37.59%), *A. niger* (33.49%) minimum percent inhibition *Rhizopus* spp. (11.51%).

However, inhibition of the radial growth in the *Pseudomonas* spp. of the pathogens *F. oxysporum* f. sp. *ciceri* (45.08%) was recorded the maximum inhibition when compared to the other pathogens, whereas, *R. bataticola* (35.87%), *A. niger* (31.75%), *Penicillium* spp. (25.18%) and *Rhizopus* spp. (13.84%) percent inhibition respectively.

Table – 10: Observation of radial colonies growth and percent inhibition of pathogens with *T. viride* and *Pseudomonas* spp. through Dual Culture technique.

Bio-agents + Pathogens	Mean of radial colony growth of pathogens and bio- agents in (mm) at 72 hrs	Percent inhibition (%) growth of pathogens over control at 72 hrs
T. viride + *R. bataticola*	25.16	41.08
T. viride + *Rhizopus* spp.	39.20	11.57
T. viride+ *A. niger*	28.13	33.49
T. viride + *Penicillium* spp.	22.30	37.59
T. viride + *F. oxysporum* f. sp.*Ciceri*	21.03	50.35
Pseudomonas spp + *R. bataticola*	27.77	35.87
Pseudomonas spp + *Rhizopus* spp.	38.16	13.84
Pseudomonas spp + *A. niger*	28.86	31.75
Pseudomonas spp + *Penicillium* spp.	26.73	25.18
Pseudomonas spp + *F. oxysporum* f. sp.*Ciceri*	23.26	45.02
R. bataticola	43.33	-
Rhizopus spp.	44.30	-
Aspergillus niger	42.30	-
Penicillium spp	35.73	-
F. oxysporium. f. sp. *Ciceri*	42.36	-
C.D. at 5 %	**0.97**	
S.E (m)±	**0.33**	

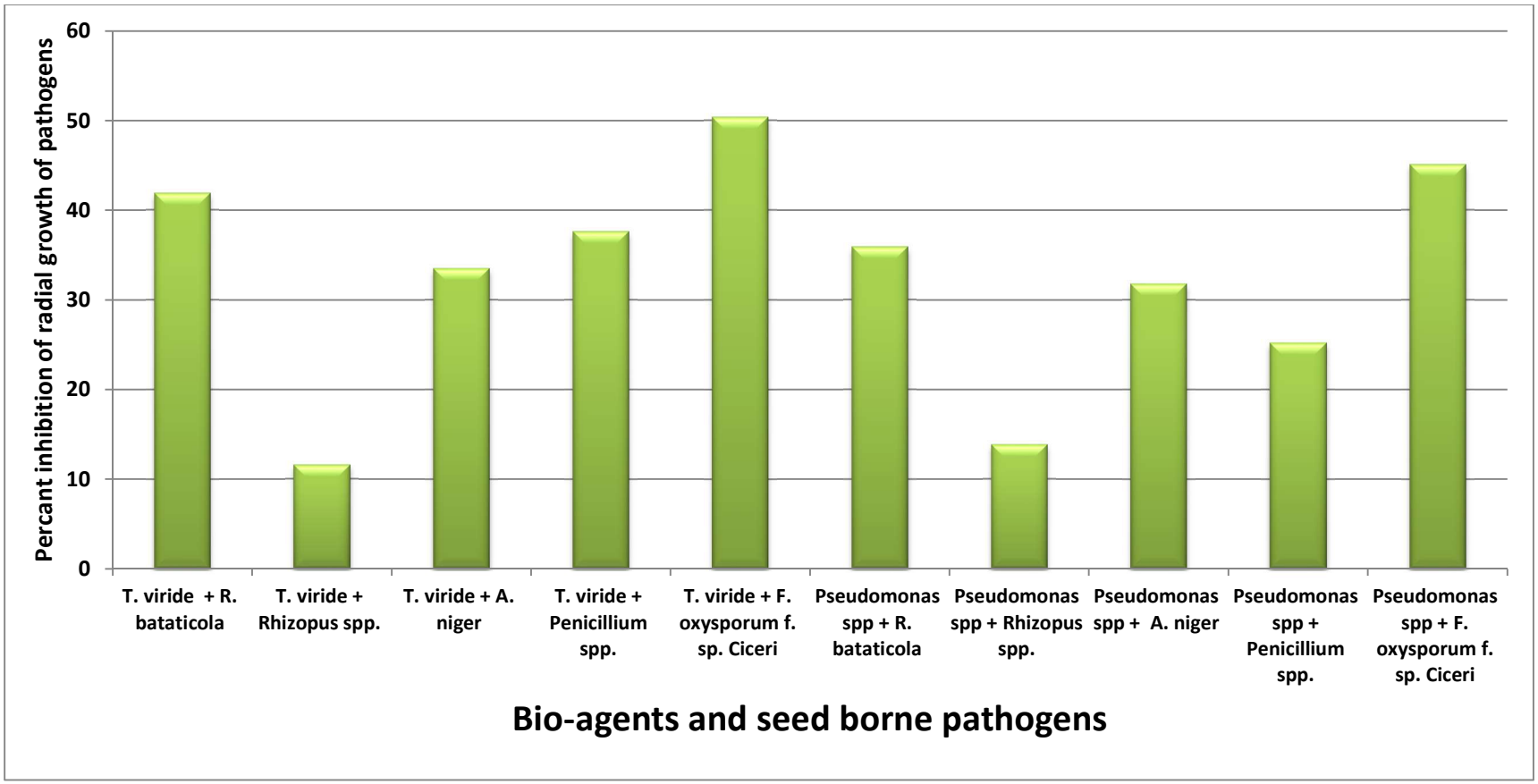
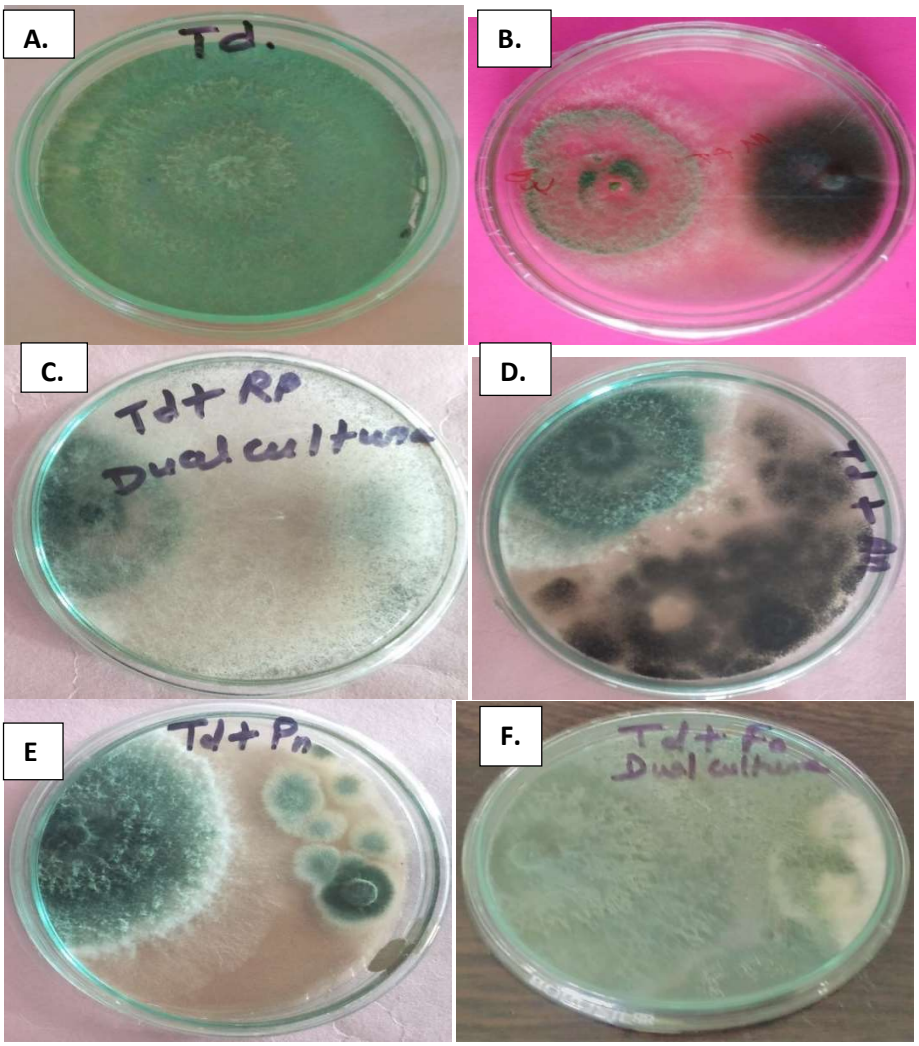


Figure-5: - Dual Culture technique of seed-borne pathogens with bio-agents.



A. ***Trichoderma viride*** **B.** ***T.viride + R. bataticola***
C. ***T. viride + Rhizopus*** **spp.** **D.** ***T. viride + A. niger***
E. ***T. viride + Penicillium*** **spp.** **F.** ***T. viride + F. oxysporum*** **f.sp.** ***ciceri***

Figure – 13: Radial colonies growth of pathogens with *T. viride* through Dual culture technique.

DISCUSSION

The present investigation Studies on seed-borne pathogens and their control included survey and collection of seed samples, detection of seed-borne pathogens, evaluation of fungicides, bio-pesticide and bio-agents against the major seed-borne pathogens.

5.1 SURVEY AND COLLECTION OF SEED SAMPLES FROM CHICKPEA GROWING AREAS.

The seed samples of chickpea collected from different parts of block Tikamgarh. The samples collected randomly from the chickpea field. 10 samples were collected from each field during survey by using quadrate sampling technique.

The collected seed samples were labeled and stored in the laboratory at the room temperature in paper bag for the further detection.

5.2 DETECTION OF SEED-BORNE MYCOFLORA.

The seed samples of chickpea collected of different parts were tested initially by employing four methods *viz.,* Dry seed inspection method, Seed washing method, Standard blotter paper method and Agar plate method.

Bretag and Mebalds (1987) also studied the seed mycoflora of chickpea in the standard blotter method and they observed several surface-borne saprophytes *Alternaria alternata, Aureobasidium pullulans, Cladosporium* spp., *Penicillium* spp., *Rhizopus stolonifer*, *Stemphylum* spp. *Vlocladium atrum* and pathogens like *ascochyta pisi, Botrytis cineria*, *Fusarium oxysporum* and *Phoma medicoginis*. Singh *et al.* (2005) also studied the seed mycoflora of chickpea by using standard blotter method and agar plate method. They reported nine fungal species belonging to eight genera namely *Alternaria alternata*, *Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme, Helminthosporium sativum*, *Mucor* sp., *Penicillium notatum* and *Rhizopus nigricans*.

5.2.1 Seed health testing methods

Examination of seed health testing method reaveld the presence of the pathogens like *R. bataticola, Rhizopus* spp*., A. niger, F. oxysporum* and *Penicillium* spp. Several criteria are involved in selecting a suitable procedure for the detection of different fungi in seeds. The primary criterion being its capacity to reveal the fungi in maximum percentage, another is versatality and capacity to reveal a wide range of pathogens. Keeping these two points in view, a study was conducted to compare the four routine seed health testing methods.

5.2.1.1 Dry seed inspection method

In the present investigation among the seed samples collected from different blocks, Baldevgarh seed sample showed the maximum infection followed by Jatara and Palera. This might due to the favorable microclimatic condition of Baldevgarh like soil type, soil moisture and soil temperature.

However the maximum morphological characteristics *i.e.,* mechanical damage and unfilled or chaffy grain were identified through dry seed inspection method, where as the spotted and discoloration type seed samples were identified in minimum number.

5.2.1.2 Seed washing method

Spore morphology of the fungus presence of outside the seed were observed through seed washing method was recorded the highest in the Jatara block, moderate the spore morphology was observed in Tikamgarh and Palera.

Among the seed borne pathogens in the *Rhizoctonia bataticola* is present in the block of Tikamgarh, Prithvipur and Palera followed by *Rhizopus* spp. which was present in Jatara and Prithvipur, *Aspergillus niger* are present in all the block except Prithvipur, *Penicillium* spp. which was observed in Baldevgarh, Jatara, Nivari and Prithvipur and *F. oxysporum* f. sp. c*iceri* which was absent in all blocks in Tikamgarh district. These findings are inconcorrance with the Chaithra (2009).

5.2.1.3 Standard blotter paper method

Through standard blotter paper method, highest numbers of colonies were showed for *F. oxysporum* f. sp. *ciceri,* followed by *A. niger R. bataticola, Rhizopus* spp. while the least number of colonies were showed for *Penicillium* spp. These finding are supported by Mailem (2013).

However the maximum numbers of colonies were recorded in Tikamgarh block followed by Nivari, Baldevgarh, Prithvipur, Palera while the minimum number of colonies were recorded in Jatara block.

5.2.1.4 Agar plate method

The maximum numbers of colonies were detected through agar plate method with PDA for *A. niger* followed by *F.oxysporum* f. sp. *ciceri, R. bataticola and Rhizopus* spp. while the least number of colonies were detected for *Penicilium* spp.

Among the blocks, the maximum numbers of colonies were detected in Tikamgarh followed by Baldevgarh, Prithvipur, Palera, Nivari while the minimum number of colonies were detected in Jatara. These results were also reported by Paul (1989), Patil *et al.* (2012) and Padmaja *et al.* (2015).

5.3. EVALUATION OF FUNGICIDE, BIO-AGENTS AND BIO-PESTICIDE AGAINST MAJOR SEED BORNE PATHOGENS OF SEED *IN-VITRO.*

5.3.1 Evaluation of fungicides and bio-pesticide (Neem extract).

Efficacy of four fungicides Carbendazim (0.2%), Carboxin (0.2%), Mancozeb (0.2%), Carbendazim (0.1%) + Carboxin (0.1%) and neem extract (5%) were evaluated against all seed borne pathogens adopting poisoned food technique.

Among the different fungicides and bio-pesticide used in the *in-vitro* condition, the superiority in controlling the inhibition of seed borne pathogens was managed by carbendazim + carboxin. These results are also repoted by Soma *et al. (*2008), Vinit *et al* (2009) and Somu *et al* (2014).

5.3.2. Evaluation of bio-agents.

The antagonistic micro-organism *viz., T. viride* and *Pseudomonas* spp. were showed the highest growth inhibition test pathogen *F. oxysporum* f. sp. *ciceri* (50.35% and 45.08%). These finding are in concurrence with the Rajput *et al.* 2010, Damaram 2012.

SUMMARY, CONCLUSION AND SUGGESTION FOR FURTHER WORK

The present investigation, **"Studies on seed-borne pathogens of chickpea and their control"** included survey and collection of seed samples, detection of seed-borne pathogens, and evaluation of fungicides, bio-pesticide and bio-agents against the major seed-borne pathogens.

6.1 Survey and collection of seed samples from chickpea growing areas.

The seed samples of chickpea collected from different parts of block Tikamgarh. The samples collected randomly from the chickpea field. 10 samples were collected from each field during survey by using Quadrate sampling technique.

The collected seed samples were labeled and stored in the laboratory at the room temperature in paper bag for the further detection.

6.1.1 Detection of seed-borne mycoflora.

The seed samples of chickpea collected of different parts were tested initially by employing four methods *viz.,* Dry seed inspection method, Seed washing method, Standard blotter paper method and Agar plate method.

6.1.2 Seed health testing methods

Examination of seed health testing method revealed the presence of the pathogens like *R. bataticola, Rhizopus* spp*., A. niger, F. oxysporum* f. sp. *ciceri* and *Penicillium* spp.

6.1.3 Dry seed inspection method

In the present investigation among the seed samples collected from different blocks, Baldevgarh seed sample showed the maximum infection followed by Jatara and Palera.

However the maximum morphological characteristics *i.e.,* mechanical damage and unfilled or chaffy grain were identified through

Dry seed inspection method, where as the spotted and discoloration type seed samples were identified in minimum number.

6.1.4 Seed washing method

Spore morphology of the fungus present on outside of the seed were observed through seed washing method was recorded the highest in the Jatara block.

Among the seed borne pathogens in the *R. bataticola* is present in the block of Tikamgarh, Prithvipur and Palera followed by *Rhizopus* spp. which was present in Jatara and Prithvipur, *Aspergillus niger* are present in all the block except Prithvipur, *Penicillium* spp. which was observed in Baldevgarh, Jatara, Nivari and Prithvipur and *F. oxysporum* f. sp. *ciceri* which was absent in all blocks in Tikamgarh district.

6.1.5 Standard blotter paper method

Through Standard blotter paper method, highest numbers of colonies were showed for *F. oxysporum* f. sp. *ciceri,* followed by *A. niger R. bataticola, Rhizopus* spp. while the least number of colonies were showed for *Penicillium* spp.

However the maximum numbers of colonies were recorded in Tikamgarh block followed by Nivari, Baldevgarh, Prithvipur, Palera while the minimum number of colonies were recorded in Jatara block.

6.1.6 Agar plate method

The maximum numbers of colonies were detected through Agar plate method with PDA for *A. niger* followed by *F. oxysporum* f. sp. *ciceri, R. bataticola and Rhizopus* spp. while the least number of colonies were detected for *Penicilium* spp.

Among the blocks, the maximum numbers of colonies were detected in Tikamgarh followed by Baldevgarh, Prithvipur, Palera, Nivari while the minimum number of colonies were detected in Jatara.

6.2. EVALUATION OF FUNGICIDE, BIO-AGENTS AND BIO-PESTICIDE AGAINST MAJOR SEED BORNE PATHOGENS OF SEED *IN-VITRO.*

6.2.1 Evaluation of fungicides and bio-pesticide.

Efficacy of four fungicides Carbendazim (0.2%), Carboxin (0.2%), Mancozeb (0.2%), Carbendazim (0.1%) + Carboxin (0.1%) and neem extract (5%) were evaluated against all seed borne pathogens adopting poisoned food technique.

Among the different fungicides and bio-pesticide used in the *in-vitro* condition, the superiority in controlling the inhibition of seed borne pathogens was noticed by Carbendazim + Carboxin.

6.2.2 Evaluation of bio-agents.

The antagonistic micro-organism *viz., T. viride* and *Pseudomonas* spp. were showed the highest growth inhibition test pathogen *F. oxysporum* f. sp. *ciceri* (50.35% and 45.08%).

Conclusion

The seed samples of chickpea collected from different parts of block Tikamgarh were labeled and stored in the laboratory at the room temperature in paper bag for the further detection by employing four methods *viz.,* dry seed inspection method, seed washing method, standard blotter paper method and Agar plate method.

Examination of seed health testing method revealed the presence of the pathogens like *R. bataticola, Rhizopus* spp*., A. niger, F. oxysporum* and *Penicillium* spp. Through dry seed inspection method maximum morphological characteristics *i.e.,* mechanical damage and unfilled or chaffy grain were identified. Through standard blotter paper method, highest number of colonies was showed for *F. oxysporum* f. sp. *ciceri*. The maximum numbers of colonies were detected through agar plate method with PDA for *A. niger.*

The antagonistic micro-organism *viz., T. viride* and *Pseudomonas* spp. were showed the highest growth inhibition test pathogen *F. oxysporum* f. sp*. ciceri* (50.35% and 45.08%).

6.3 Suggestions for further work.

On the basis of the presence study, the following suggestions may be implemented for the further study.

1. The efficacy of the fungicides and bio-agents may be tested at different dosages.
2. The inhibition of the seed-borne pathogens may be performed based on the *in-vitro* conditions.

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APPENDIX

Appendix - I ANOVA of Evaluation of bio-agents through Dual Culture technique

Source of variation	Degree of Freedom	Mean sum of squares
Treatment	14	214.09
Error	30	0.33
Total	44	-

Appendix - II ANOVA of Evaluation of Fungicides and Bio-pesticide through Poisoned Food technique

Source of variation	Degree of Freedom	Mean sum of squares				
		R. bataticola	***A. niger***	***F. oxysporum* f. sp.*ciceri***	***Penicillium* spp.**	***Rhizous* spp.**
Treatment	5	1197.7	765.3	677.9	670.9	644.2
Error	12	0.06	0.16	0.09	0.22	0.26
Total	17	-	-	-	-	-