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Effect of fungicide (Bavistin, Mancozeb) and biofungicide (Cow urine, Azadirachta Indica, Curcuma Longa, Trichoderma viride) on Seed-Borne Mycoflora and Growth of chilly (Capsicum Annuum L.)

Impana, K.V[1], Thanushree, C. G[2], Sindhu, G. M[3]

[1,3]PG Department of Botany, JSS college of Arts, Commerce and Science, Mysore, Karnataka
[2]Lecturer, Department of Biology, SIR MV PU College, Davangere, Karnataka

Abstract:
Chilly is one of the major crop productions in tropical and sub-tropical regions. Present research was aimed to study the effect of fungicide and biofungicide on seed borne mycoflora and growth of chilly (*Capsicum annuum* L.). Chilly seeds were treated with biofungicide (Cow urine, *Azadirachta indica, Curcuma longa, Trichoderma viride*) and distilled water treated seeds were used as negative control and fungicide (Bavistin and Mancozeb) treated seeds as well as hybrid seeds were used as positive control. Standard blotter method was used to assess seed borne mycoflora, greenhouse studies viz., vigour index, chlorophyll test, thin layer chromatography, phenol test were conducted to study growth promoting ability of the treatments. In blotter method, six different seed borne fungi were identified namely, *Alternaria* sp., *Colletotrichum* sp., *Cladosporium* sp.1, *Cladosporium* sp.2, *Curvularia* sp, *Rhizopus* sp. green house studies revealed that highest growth was recorded in hybrid seeds and protection from fungal pathogens was observed in fungicide (Bavistin and Mancozeb) treated seeds and least in control (distilled water) seeds. Among biofungicide treated seeds *Trichoderma viride* stands best followed by Neem, cow urine, Turmeric treated seeds. Which is in par with the growth promotion offered by hybrid seeds and protection offered by fungicide treated seeds. Our results highlight that use of hybrid seeds or fungicides can either promote growth or offer protection respectively but use of bio fungicide will enhance overall growth and also offer protection to chilly seeds.

Keywords: Capsicum annuum L., fungicide, Seed borne fungi, Cow urine, Azadirachta indica, Curcuma longa, Trichoderma viride.

INTRODUCTION

Chilly belongs to the genus *Capsicum* under the Solanaceae family widely grown in almost all areas and seasons. Despite having a high nutritional content, being well received by customers, and having a wide spectrum of genetic variability (Sarkar *et al*., 2009). Leading producers of chilies include China (8.6%), Peru (4.74%), India (near to 39.78% of global production), Pakistan, Morocco, Mexico, and Turkey are additional significant producers and exporters of chilies. The top ten nations that produce chilies include

India, China, Thailand, Pakistan, Ghana, Ethiopia, Peru, Bangladesh, and others. According to the FAO, chilies were grown in an area of about 19.74 lakh ha. In contrast to nations like Cape Verde, Jamaica, and Morocco, where the yield levels are higher than 10 t/ha, India only produces 1.75 t/ha (Devi *et al*., 2016). Chilly suffers from many diseases caused by fungi, bacteria, viruses, nematodes, and also biotic stresses. Fungal disease plays a vital role in reducing the germination of chilly. Seed treatment process of treating seeds by any physical, chemical, or biological harmful seed-borne organisms or to protect the seeds against infection (Afrayeem and Chaurasia, 2017). *Alternaria solani, Botrytis cineria, Fusarium, Colletotrichum, Cercospora capsica* and *Scferotinia sclerotiorum* are the most significant seed-borne fungi in chilly (Nawaz *et al*., 2019).

Seed is a key component of crop production and microorganisms that cause a variety of diseases that spread through seeds significantly reduce yields. The work has been undertaken to isolate and identify seed-borne mycoflora of locally accessible seed in certain Abbottabad its ecological importance and the losses caused by seed-borne fungi (Nawaz *et al*., 2019). Among, diseases that are spread by seeds, primarily fungus, are crucial to the emergence of illness. Farmers must deal with considerable losses as a result of major seed-borne fungus infecting their plants. These infections can begin with germination of the seed, seedling growth in the nursery, and mature plants in the field, and continue through harvest, fruit production, and seed storage. It has been documented that seed-borne diseases have caused significant crop losses. Since an infected seed is less viable, has low germination, decreased vigor, and decreased yield, seed health and quality are a crucial factor in the prevention of diseases (Mohanto *et al*., 2019).

Seeds are a highly efficient method for spreading plant infections across great areas because of their high mobility. Numerous instances of the global spread of land diseases as a result of the importation of seeds that were pathogen-contaminated or infected may be found in agricultural literature (Abdulsalaam and Shenge, 2011). Almost 90% of crops are propagated from seeds, yield and losses of sustainable crop production are greatly influenced by the quality of the seeds, which are a key element of agriculture strategy. Hence, the use of sensitive techniques for the detection, identification, and differentiation of the pathogen present in seeds is considered to be of the utmost importance. Microorganisms that are present in seeds can harm a plant's ability to survive, grow, and reproduce. The Lack of effective control measures facilitates the spread of the virus through seeds to unaffected areas, which results in economic loss (Kumar *et al.,* 2020).

Pathogens found in seeds pose a significant hazard to seedling establishment. Pathogens can survive for a long time, can be introduced into new environments, and can spread widely when they are closely associated with seeds. Due to characteristics including large populations of vulnerable plants, high relative humidity, high temperatures, and overhead irrigation, plant diseases have a high chance of causing major economic losses in greenhouse environments. Exclusion is the most efficient disease management technique in these circumstances and it is performed by screening and removing infected seed lots before planting using seed detection assays (Walcott, 2003).

Typically, infections brought on by fungi are more destructive than those brought on by other pathogens. Various fungi infect chilly plants and produce various diseases. These fungi can occasionally produce similar symptoms and be confused with one another. There are some pre-harvesting fungal diseases such as; Anthracnose, *Cercospora* (frog eye) leaf spot, Charcoal rot, *Choanephora* blight (wet rot), Damping-off, root rot, Downy mildew, *Fusarium* stem rot, *Fusarium* wilt, Grey leaf spot, Grey mold, *Phytophthora* blight, Powdery mildew, Southern blight, *Verticillium* wilt and White mold are grown in chilly crop (Hussain and Abid, 2011).

The need for adopting biological approaches as an alternative eco-friendly disease management strategy has been highlighted by the growing knowledge of fungicide-related risks. Biological control strategies make use of organisms that are antagonistic to the target pathogens. Microorganisms known as endophytes are endo-symbionts that exist within plant tissue without creating any disease symptoms (Hassan *et al*., 2015). The neem tree (*Azadirachta indica*) and its chemical byproducts have been used for ages in a variety of ways for human benefit, including as an agrochemical, pesticide, food, heating source, wood, soil amendment, and an important therapeutic agent for allegedly more than 100 maladies. The *Azadirachta indica* belongs to the Meliaceae family, sometimes known as the mahogany family. Neem populations are diverse in all ways, responding strongly to variations in soil and climate (Hassan *et al*., 2015). As a member of the ginger family (Zingiberaceae), turmeric (*Curcuma longa* L.) is one of the most common rhizomatous spices used in alternative medicine all over the world. The entire plant has a pleasant perfume, but the most important products are the underground rhizomes, whether they are raw or processed. It is thought that the biological effects of the active ingredient "curcumin" include anti-inflammatory, antioxidant, antitumor, antibacterial, and antiviral properties (Vinayarani and Prakash, 2018). The fungus antagonist *Trichoderma* species is frequently used to inhibit *Colletotrichum* species in chilly. Additionally, it is thought that *Trichoderma* species can successfully compete with one another for surface area, decreasing the likelihood of a pathogen infection (Kiran *et al*., 2020).

In addition to giving nutrients like potassium and compounds that are good for plants, cow urine is an inexpensive input that is simple for rural producers to obtain. It is well recognized to have a positive impact on germination, and growth parameters such as plant height, leaf area, and number of leaves; additionally, it has a positive effect on yield parameters such as tiller number, grain weight, and crop yield. This has been explained by the presence of physiologically active compounds in cow urine, such as nutrients, trace elements, and growth regulators (Tiwari *et al*., (2018).

In comparison to other systemic fungicides, Bavistin (carbendazin 50%WP) 0.1%, Plantvax (oxycarboxin), and vitavax (carboxin) were found to be effective as they reduced the disease by 80.84% and by monitoring the spore germination of *C. truncatum*, respectively. Propiconazole showed the highest level of inhibition of *in vitro* mycelial growth, biomass production, sporulation, and spore germination at concentrations as low as 0.1 mg/ ml. Other systemic fungicides from the triazole group, such as difenoconazole and benzimidazole, have also been used in both pre and post-harvest management of chilly anthracnose. Other dithiocarbamate fungicides, such as Bordeaux mixture (0.5 or 1%) of a copper sulfate fungicide and Mancozeb (0.2%), ziram (0.1%), copper oxychloride fungicide (Blitox 50), were shown to be efficient in controlling this disease (Kiran *et al*., 2020).

MATERIAL AND METHODS

Experimental site: The experiment was conducted at laboratory, department of Botany, JSS college of Arts, Commerce and Science, Mysore.

Experiment period: The experiment was conducted during the period from May to July, 2023.

Collection of seed sample: Seeds were collected from different agro shops in Mysore. The samples were brought directly to the laboratory and kept in polythene bags and stored in the refrigerator, till the seeds were used for the subsequent studies.

Selected seeds: Seeds of chilly were selected to know the effect of seed-borne pathogen.

Seed treatment: Susceptible chilly seeds were treated with bio fungicides (Cow urine, Neem, Turmeric, *Trichoderma viride*), and Untreated/ Distilled water treated seeds were used as negative control. Bavistin

and Mancozeb treated seeds and hybrid seeds were used as a positive control.

Preparation neem extract: Fresh leaves of neem obtained from Shrirangapatna, near Ganjam, were washed thoroughly with tap water. The fresh leaves were dried in room temperature and finely powdered. 4 grams of the neem leaf powder was put into a beaker and 40 ml of distilled water was added and stirred thoroughly with a glass rod to obtain an extract with a concentration of 2 millimolar (Gyasi *et al*., 2020).

Preparation of turmeric extract: Fresh turmeric rhizome was obtained from Chamarajanagar. The turmeric was dried in at the room temperature and after drying the turmeric was finely powdered. 4 grams of turmeric powder was put into a beaker and 40 ml of distilled water was added and stirred thoroughly with a glass rod to obtain an extract with concentration of 2 millimolar.

Preparation of *Trichoderma viride* sample: *Trichoderma viride* was obtained from the agro shop from Mysore. *Trichoderma viride* solution was prepared by following the manufacturer's recommendation. About 4 grams of the *Trichoderma viride* powder was taken and dissolved in 40 ml of distilled water was added and stirred thoroughly with a glass rod to obtain an extract with a concentration of 2 millimolar.

Preparation of cow urine sample: the Cow urine sample was prepared by following the manufacturer's recommendation. About 1 ml of cow urine was measured and put into a beaker and 10 ml of distilled water was added and stirred thoroughly with a glass rod to obtain an extract with a concentration of 2 millimolar (Gyasi *et al*., 2020).

Preparation of Bavistin solution: Bavistin was obtain by agrochemical shop in Mysore. Bavistin solution was prepared by following the manufacturer's recommendation. About 0.0010 grams of the Bavistin fungicide powder was taken and dissolved in 10 ml of distilled water.

Preparation of Mancozeb solution: Mancozeb was obtained by agrochemical shop from Mysore. Mancozeb solution was prepared by following the manufacturing's recommendation. About 0.0010 grams of the Mancozeb fungicide powder was taken and dissolved in 10 ml of distilled water (Gyasi *et al*., 2020).

A. Assessment of seed-borne fungi in chilly seeds treated with fungicide (Bavistin and Mancozeb) and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) by standard blotter method

To detect the seed-borne pathogen associated with the seeds in the samples, the blotter method was used following the International rules for Seed Testing Association (ISTA, 2001). In this method, two layers of blotter paper were soaked in distilled water and placed at the bottom of 9 cm diameter plastic Petri dishes. This blotter method is conducted with the seeds treated with distilled water, fungicide (Bavistin and Mancozeb), and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) and hybrid seeds. Four hundred seeds were used for the blotter test for detection of seed-borne fungi. Chilly seeds were platted, 25 seeds (15 in outer, 9 in middle, 1 in centre) per plate. The Petri dishes with seeds were then incubated for 12/12 hours alternating light and darkness in the incubation room for seven days at 20±2°C. After incubation, fungi developed on each seed were examined under a Stereo-binocular microscope. Data were also recorded on seed germination (%), seed infection (%), and seed borne pathogen. Each individual incubated seed was observed under Stereo-microscope at 40x magnification to record the incidence of seed-born fungi. Most of the associated pathogens were detected by their growth character on the incubated seed on Blotter paper following the keys outlined by Ramnath *et al*. (1970), and Gyasi *et al*. (2020).

B. Effect of fungicide (Bavistin and Mancozeb) and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) on *in vitro* seed germination and vigour index

The brown germination paper is sterilized before use and soaked in distilled water. Seeds treated with distilled water, fungicide (Bavistin and Mancozeb), and Bio fungicide (Cow urine, Neem, Turmeric,

Trichoderma viride) and hybrid seeds. Over 50 seeds from each treatment are placed on a sterilized paper towel (eqvi distance on germination paper) and another portion of the paper towel is placed on the first off so that seeds are held in position. The germination paper is rolled and kept directly in the trays for germination with water maintained in the germination tray to avoid decaying germination paper. After the 7 days of incubation, germination paper was unrolled and the number the seeds germinated was calculated and the seedling vigor was analysed. The Shoot length and root length of the individual seedling is measured to determine the vigor index (Bhagora *et al*., 2019).

Germination percentage: Germination percentage was estimated by the below formula given by Rehman *et al*. (1998).

Germination % = number of seed germinated/ total no. of seeds × 100

Vigour index: Vigor index was calculated using the following formula suggested by (Baki *et al.,* 1973).

Vigour index = germination % × (mean root length + mean shoot length)∗

(∗ Indicate that root length and shoot length should be in cm).

C. Effect of fungicide (Bavistin and Mancozeb) and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) on chilly seeds under greenhouse conditions

The experiment was conducted under germination conditions, chilly plants were subjected to different treatments. susceptible chilly seeds were treated with bio fungicides (Cow urine, Neem, Turmeric, *Trichoderma viride*), and Untreated/ Distilled water treated seeds were used as a negative control. Bavistin and Mancozeb treated seeds and hybrid seeds were used as positive control. For the pot culture experiment, pots were filled with soil. The seeds were treated as detailed in the laboratory experiment and four seeds were placed at a depth of 1-2 cm in each of the pots. The pots were watered daily for up to 21 days. The weeds were uprooted whenever seen. The final count was made on the 21th day and only normal seedlings were considered for germination testing. Normal seedlings were selected randomly at the time of final count for observations on shoot length, root length, average no. of leaves, branches, and total seedling length (Anderson *et al.*, 1973; Gunawardena *et al*., 2014; Reddy *et al*., 2018).

D. Estimation of total chlorophyll from the seedling treated with fungicide (Bavistin and Mancozeb) and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*)

Total chlorophyll was estimated from the leaf by the method of Witham *et al*. (1971). Briefly, 1g of finely cut fresh leaf was crushed in pre-chilled mortar and pestle with 20 ml 80% chilled acetone and centrifuged at 5000 rpm for 5min and the supernatant was collected. The process was repeated until the residue was colourless. Volume was made up to 100 ml with 80% chilled acetone. The absorbance of the solution was noted at 645 nm and 663 nm in a spectrophotometer against the solvent blank. The amount of chlorophyll-a, chlorophyll-b, and total chlorophyll were calculated in mg/ g fresh weight according to the following equation.

1. Chlorophyll a = 12.7 × (D-663) - 2.69 × (D-645) × V/1000 × W
2. Chlorophyll b = 22.9 × (D-645) - 4.68 × (D-663) × V/1000 × W
3. Total chlorophyll = 20.9 × (D-645) + 8.02 × (D-663) × V/1000 × W

Where,

D - 645 = Optical density at 645 nm

D – 663 = Optical density at 663 nm

V = Final volume of 80% acetone chlorophyll extract in ml

W = Fresh weight in g of a corresponding amount of fresh leaves used in the extraction of chlorophyll for all chemical analysis purposes sterilized oven-dried glassware and double distilled water were used throughout the experiment (Nayak *et al*., 2016).

E. Plant pigment thin layer chromatography

Collect 1g fresh green leaves, grind well with acetone, and filter to extract plant pigments. Fill a capillary tube by placing it in the leaf extract, apply extract 2 cm above the lower edge on the strip of TLC plate by quickly touching the end, allow to dry, repeat several times to make a concentrated dot of extract then carefully place the TLC plate in the coupling jar containing petroleum ether and acetone (9:1). The TLC plate should sit on the bottom of the chamber and be in contact with the solvent, allow the TLC plate to develop (separation of pigment) for approximately 10 minutes. Remove it from the chamber when the solvent is approximately 1.0 cm from the top of the TLC plate, mark the level of the solvent travelled, and calculate and record the Rf valves (Sestak, 1959).

Distance moved by the $RF = \frac{\text{nt}}{\text{Distance moved by the solvent}}$

F. Estimation of total phenol from the seedling treated with fungicide (Bavistin and Mancozeb) and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*)

The total phenolic content of plant extracts was estimated by the Folin- Ciocalteau (FC) method. 1ml of the plant extracts was taken in test tubes and 1.0 mL of FC reagent was added, after 3-5 min, 2.0 mL of a sodium carbonate solution was added and the mixture was allowed to stand for 10 minutes in a water bath. After the incubation period the absorbance was taken at 660 nm in a spectrophotometer. Gallic acid is used as standard. The concentration of total phenolics was expressed in terms of mg GAE/ g gallic acid equivalents (Volluri, 2011).

RESULT

Laboratory and pot experiments were conducted to examine the effect on susceptible chilly seeds that were treated with bio fungicides (Cow urine, Neem, Turmeric, *Trichoderma viride*), Untreated/ Distilled water treated seeds were used as negative control. Bavistin and Mancozeb treated seeds and hybrid seeds were used as positive control on (1) Detection of seed-borne fungi in chilly seeds treated with fungicide (Bavistin and Mancozeb) and fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) by Standard blotter method. (2) Effect of fungicide (Bavistin and Mancozeb) and fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) on in vitro seed germination and vigor index. (3) Effect of fungicide and biofungicide on chilly seeds under Greenhouse conditions. (4) Estimation of Total chlorophyll from the seedlings treated with fungicide and biofungicide. (5) Plant pigment by thin layer chromatography. (6) Estimation of total phenol from the seedlings treated with fungicide and biofungicide.

A. Assessment of seed-borne fungi in chilly seeds treated with fungicide (Bavistin and Mancozeb) and Bio fungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) by Standard blotter method

Effect of fungicide and biofungicide on seed-borne mycoflora of chilly. The seeds were treated with different treatments of fungicide and biofungicide and allowed for 7 days of incubation and observed for the development of different mycoflora. The control seeds showed a relatively higher amount of mycoflora than other treated seeds. Treatment with fungicides (Bavistin and Mancozeb) resulted in the lesser occurrence of the mycoflora. Hybrid seeds show a lesser occurrence of mycoflora. Relatively, in the

biofungicide-treated seeds of *Trichoderma viride* was found better occurrence of the mycoflora over the Neem, turmeric, and cow urine treated seeds (Fig. 1, 2a, and 2b).

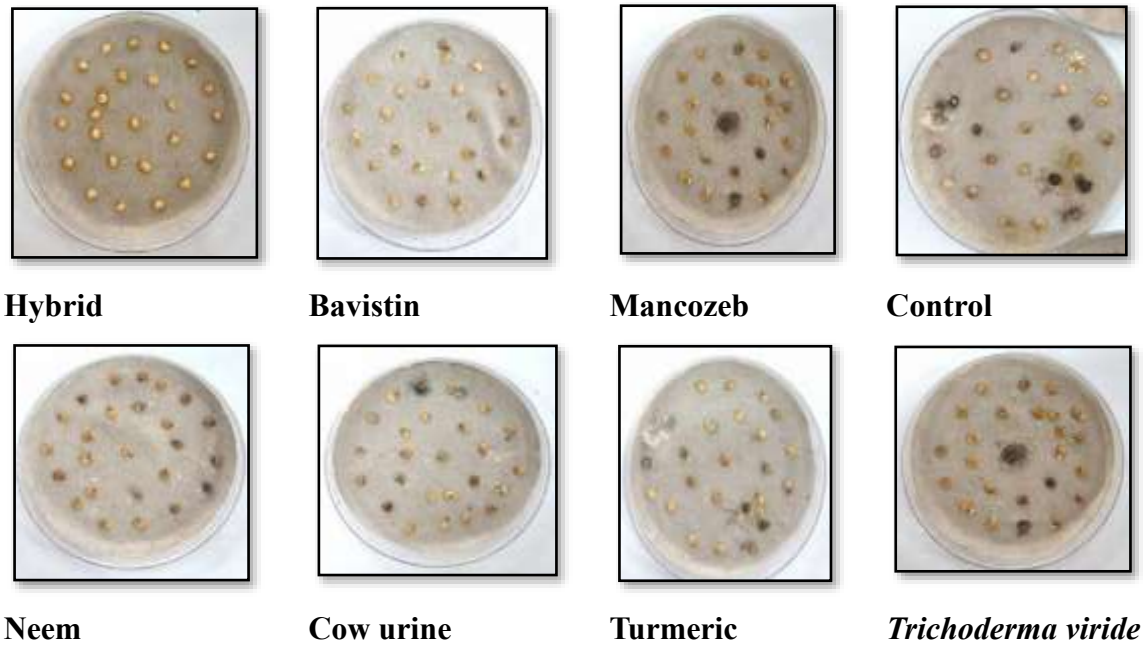
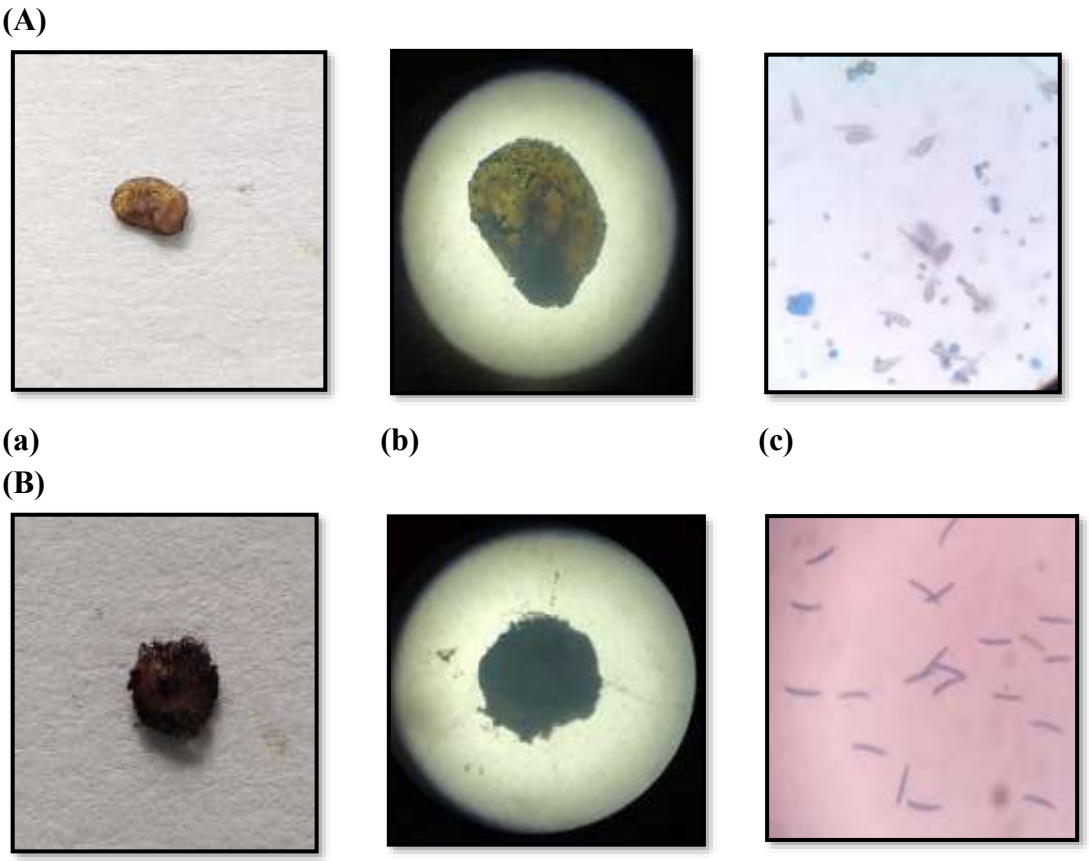
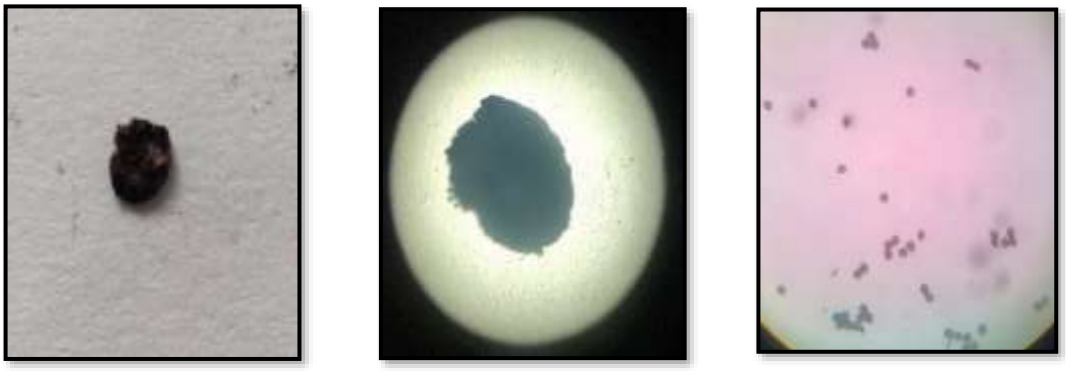


Fig. No. 1: Seed borne fungi in hybrid, Seed-borne fungi in control, Seed borne fungi in fungicide (Bavistin and Mancozeb), Seed borne fungi in biofungicide (Cow urine, Neem Turmeric, *Trichoderma viride*).



(a) **(b)** **(c)**

(C)



(a) **(b)** **(c)**

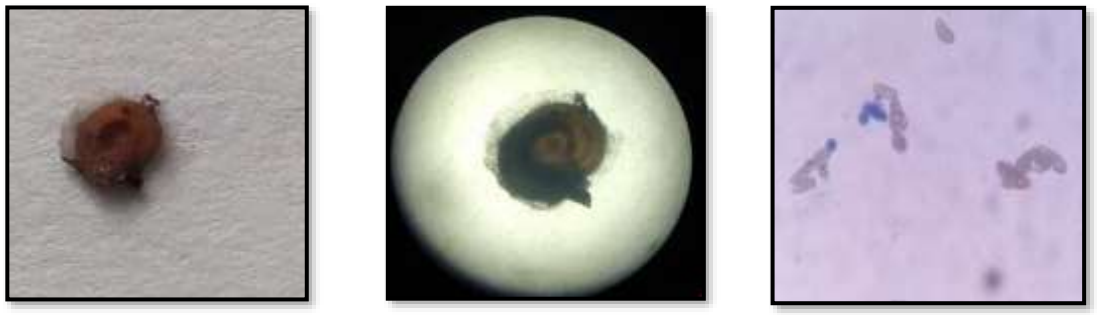
Fig. No. 2a: Microscopic observation of seed-borne fungi

A. (a) on seed colony of *Alternaria*. **(b)** *Alternaria* colony under stereo microscope. **(c)** *Alternaria* spp. under microscope (40x).

B. (a) on seed colony of *Colletotrichum.* **(b)** *Colletotrichum* colony under stereo microscope. **(c)** *Colletotrichum* spp. under microscope (40x).

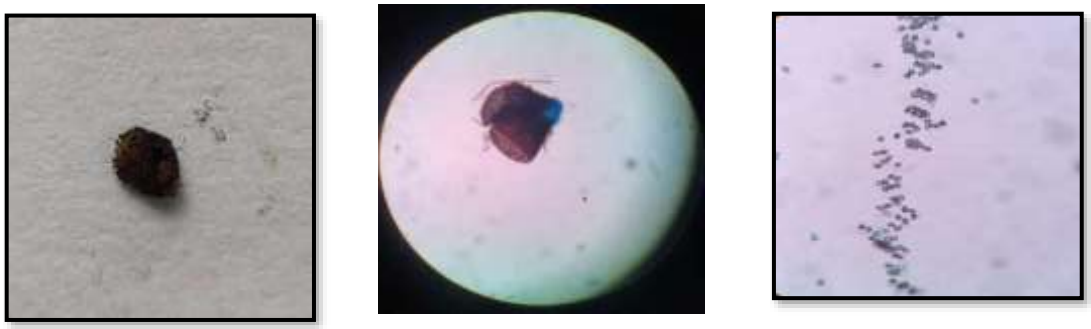
C. (a) on seed colony of *Cladosporium.* **(b)** *Cladosporium* colony under stereo microscope. **(c)** *Cladosporium* spp. 1 under a microscope (40x).

(D)



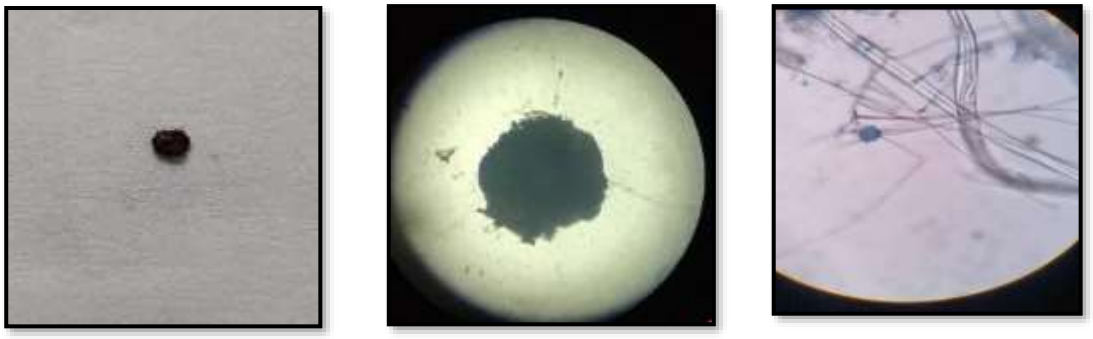
(a) **(b)** **(c)**

(E)



(a) **(b)** **(c)**

(F)



(a) **(b)** **(c)**

Fig. No. 2b: Microscopic observation of seed-borne fungi

D. (a) on seed colony of *Curvularia*. **(b)** *Curvularia* colony under stereo microscope. **(c)** *Curvularia* spp. under microscope (40x).

E. (a) on seed colony of *Cladosporium*. **(b)** *Cladosporium* colony under stereo microscope. **(c)** *Cladosporium* spp. 2 under a microscope (40x).

F. (a) on seed colony of *Rhizopus*. **(b)** *Rhizopus* colony under stereo microscope. **(c)** *Rhizopus* spp. under microscope (40x).

Table 1: Showing total no. of seeds infected and incident (%) of infection of different seed mycoflora in different treatment

Seed myco flora	hybrid		fungicide				SDW Control		biofungicide							
			Bavistin		Mancozeb				Neem		Turmeric		Cow urine		*T. viride*	
	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection (I)	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection
Alternaria sp.	0	0	0	0	0	0	0	0	4	16%	4	16 %	3	12 %	0	0

Seed myco flora	hybrid		fungicide				SDW Control		biofungicide							
			Bavistin		Mancozeb				Neem		Turmeric		Cow urine		*T. viride*	
	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection (I)	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection	Total no seed infected	% of infection
Alternaria sp.	0	0	0	0	0	0	0	0	4	16%	4	16 %	3	12 %	0	0
Colletotrichum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium sp.	0	0	0	0	2	8%	9	36 %	2	8%	8	32 %	3	12 %	3	12 %
Cladosporium sp.	0	0	0	0	0	0	9	36 %	0	0	8	32 %	6	24 %	3	12 %
Curvularia sp.	6	24 %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus sp.	0	0	0	0	1	4%	5	20 %	4	16%	0	0	0	0	0	0

B. Effect of fungicide (Bavistin and Mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) on in vitro seed germination and vigor index

The 14 days of different treatments of chilly seedlings. The Hybrid seeds show the increasing in root and shoot length secondly, the Fungicide (Bavistin) and thirdly, Biofungicide (*Trichoderma viride*) followed by Fungicide (Mancozeb), Neem, cow urine, turmeric treated seeds and control-treated seeds showing the less development of root and shoot length compare to all treatments.

The effect of hybrid seeds showed percent increase in germination% (94%) compared to other treatments of Fungicide (Bavistin- 92%), Biofungicide (*Trichoderma viride*-88%), fungicide (Mancozeb- 80%), biofungicide (neem- 74%, cow urine- 70%, turmeric- 68%) and control (64%) (Table 2).

In vigor index the lowest vigor index was 147.62 observed in control seeds and fungicide (Bavistin and Mancozeb mg/l) was (352.17 and 308.13) and in treatment of Biofungicide (*Trichoderma viride*, neem, cow urine, turmeric mg/l) was (344.73, 227.39, 163.69, 150.35) and the highest increase vigor index was 395.34 observed in hybrid Chilly seedlings (Table 3).

Table 2: Effect of fungicide (Bavistin and mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) pre-treatment on germination % of the chilly seedling.

Sl. no	Treatment		No. of seeds germinated/ total no. of seeds × 100	Germination %
1	Hybrid		47/50×100	94%
2	Fungicide	Bavistin	46/50×100	92%
		Mancozeb	40/50×100	80%
3	SDW Control		32/50×100	64%
4	Biofungicide	Neem	37/50×100	74%
		Turmeric	34/50×100	68%
		Cow urine	35/50×100	70%
		T. viride	44/50×100	88%

Table 3: Showing the effect of fungicide (Bavistin, Mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) pre-treatments on vigor index of the chilly seedling.

Sl. no	Treatment		Germination %× (Root length +Shoot length)	Vigor index
1	Hybrid		94×(2.42+4.18)	395.34
2	Fungicide	Bavistin	92×(0.82+3.008)	352.17
		Mancozeb	80×(2.53+3.82)	308.13
3	SDW Control		64×(0.298+2.302)	147.62
4	Biofungicide	Neem	74×(3.467+3.026)	227.39
		Turmeric	68×(0.622+2.202)	150.35
		Cow urine	70×(1.29+2.32)	163.69
		T. viride	88×(2.41+3.89)	344.73

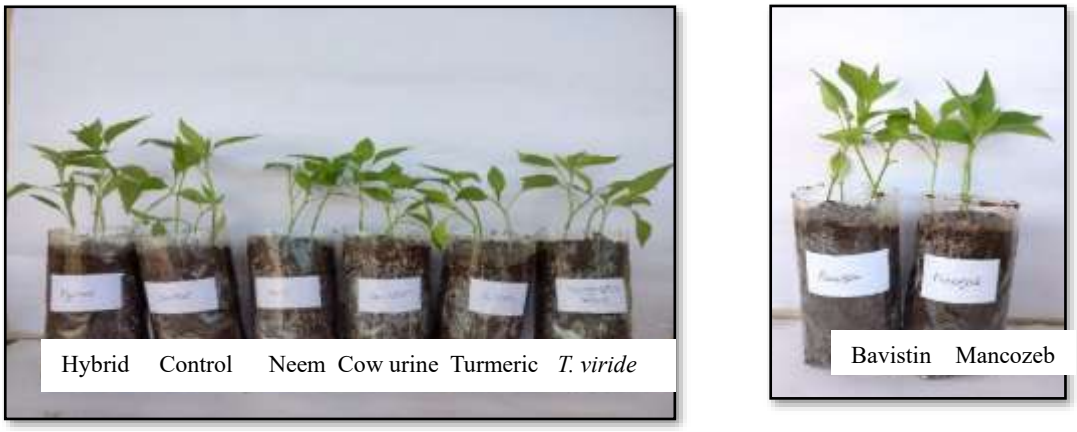
C. Effect of fungicide (Bavistin and Mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) on chilly seeds under Greenhouse conditions

Seed treatment was affected to Chilly seeds in greenhouse conditions. Where the seeds were grown and plants showed different weight, heights, root lengths, and shoot length. By observing a total number of leaves, branches, growth rate in a biofungicide (Neem, Cow urine, Turmeric, *Trichoderma viride*), fungicide (Bavistin and Mancozeb), and control seeds and hybrid seeds plants showed variation in their growth rate. The hybrid seeds show the highest growth rate compare to all treatments like fungicides (Bavistin and Mancozeb) and biofungicide (neem, cow urine, turmeric, *Trichoderma viride*). The minimum growth was observed in control seeds.

The data recorded on a number of branches produced per plant which treated with different treatments. From the data, it was observed that the number of branches produced per plant varied significantly in

chilly genotypes during the last harvest of growth. The genotypes at the last harvest stage produced maximum branches in hybrid plant and a minimum number of branches produced in control, fungicide (Bavistin and Mancozeb), biofungicide (cow urine, turmeric, neem, *Trichoderma viride*) treated plant. Such variation in the number of branches per plant may be due to genotypes characteristic of genotypes, interaction with environment, and soil factors.

In the phenol content Highest phenol content was observed in cow urine and *Trichoderma viride* and compared to all other treatments like control, neem, Bavistin, Mancozeb, and hybrid treated seeds. Leeser phenol content was observed in turmeric-treated seeds (Table 4) (Fig. 6).



(A)

Hybrid **Bavistin** **Mancozeb** **Control**

Neem **Cow urine** **Turmeric** ***T. viride***

(B)

Fig. No. 3: A. Potted plant, B. Observation of shoot length, root length

Table 04: Evaluation of fungicide (Bavistin, mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) on growth parameters of chilly

Sl. No.	Treatment		Fresh weight in g	Dry weight in g	Root length in cm	Shoot length in cm	No. of leaves	No. pf branches	Phenol in mg
1	Hybrid		0.5122	0.0798	8	18	6	3	28mg
2	Fungicide	Bavistin	0.279	0.0150	4.7	13.2	4	2	37mg
		Mancozeb	0.1763	0.0090	4.45	12	5	2	35mg
3	SDW Control		0.0862	0.0050	2.5	7.3	4	2	40mg
4	Biofungicide	Neem	0.1044	0.0061	4.28	12	4	2	40mg
		Turmeric	0.1551	0.0085	5.2	12.4	4	2	24mg
		Cow urine	0.1817	0.0096	4	13.7	4	2	49mg
		T. viride	0.394	0.0300	5.4	15	4	2	48mg

D. Estimation of Total chlorophyll from the seedlings treated with fungicide (Bavistin and Mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*)

The result of the variation of chlorophyll content (a, b, and total chlorophyll) of Chilly leaves due to the application of different treatments are given in Table 5. The both of increasing as well as decreasing were shown in total chlorophyll content was observed in different treatments.

In treatment of biofungicide (turmeric) was showing higher chlorophyll content (chlorophyll 'a' is 0.99 mg/g) (chlorophyll 'b' is 1.61 mg/g and total chlorophyll is 2.61 mg/g) and in biofungicide (neem) (chlorophyll 'a' is 0.90 mg/g, chlorophyll 'b' is 1.69 mg/g and total chlorophyll is 2.60 mg/g) and in fungicide (Bavistin) (chlorophyll 'a' is 0.81 mg/g, Chlorophyll 'b' is 1.66 mg/g and total chlorophyll is 2.48 mg/g) and in Biofungicide (cow urine) (chlorophyll 'a' is 1.01 mg/g, Chlorophyll 'b' is 1.22 mg/g and total chlorophyll is 2.24 mg/g) and in fungicide (Manozeb) (chlorophyll 'a' is 1.05 mg/g, Chlorophyll 'b' is 1.30 mg/g and total chlorophyll is 2.35 mg/g) and in hybrid (chlorophyll 'a' is 0.93 mg/g, Chlorophyll 'b' is 1.45 mg/g and total chlorophyll is 2.22 mg/g) and in control (chlorophyll 'a' is 0.80 mg/g, Chlorophyll 'b' is 1.25 mg/g and total chlorophyll is 2.05 mg/g) and lower chlorophyll content (chlorophyll 'a' is 0.68 mg/g) (chlorophyll 'b' is 1.20 mg/g and total chlorophyll is 2.04 mg/g) was observed in biofungicide (*T. viride*) treated chilly leaves (Table 05) (Fig. 4).

Table 05: Variation of chlorophyll content (a, b, total) in fungicide (Bavistin and Mancozeb) and biofungicide (Cow urine, Neem, Turmeric, *Trichoderma viride*) treated chilly leaves.

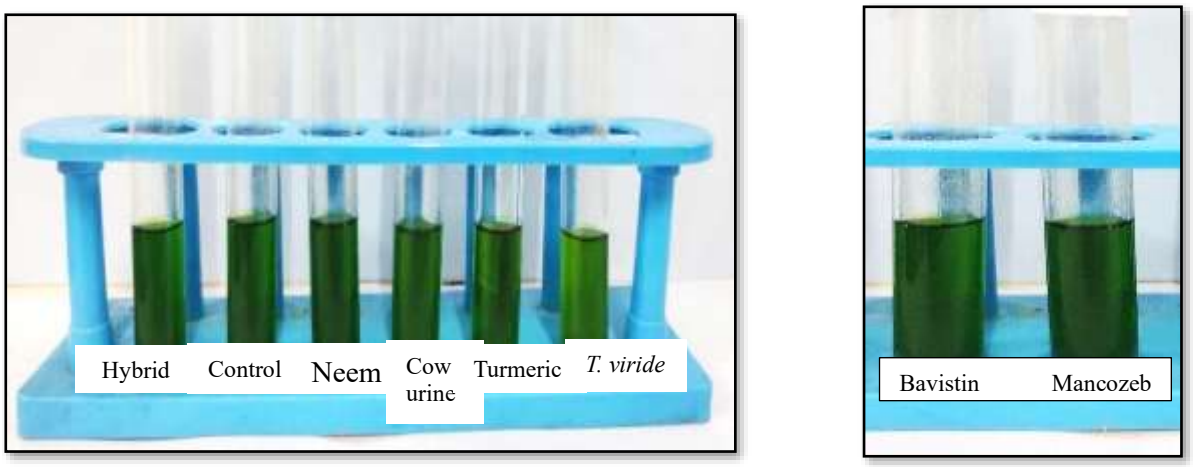
Chlorophyll contents mg/g leaf	Hybrid	Fungicide		SDW Control	Biofungicide (in mg/g)			
		Bavistin	Mancozeb		Neem	Turmeric	Cow urine	*T. viride*
Chl. a	0.93	0.81	1.05	0.80	0.90	0.99	1.01	0.68
Chl. b	1.45	1.66	1.30	1.25	1.69	1.61	1.22	1.20
Total Chl.	2.22	2.48	2.35	2.05	2.60	2.61	2.24	2.04

E. Plant pigment by thin layer chromatography:

The experiment is based on the principle of solubility in the solvents. Formation of pigments on TLC plate. This shows the chlorophyll a, b, and xanthophyll. The pigments are raised to different levels by capillary action and thus they get separated (Table 06).

Table 06: Separation of pigment by thin layer chromatography method

Sl. no	Treatment		Pigment	Description of color	Rf value	Name of the pigment
1	Hybrid		1	Grassy green	0.08	Chlorophyll a
			2	Olive green	0.12	Chlorophyll b
			3	Yellow	00	Xanthophyll
2	Fungicide	Bavistin	1	Grassy green	0.05	Chlorophyll a
			2	Olive green	0.08	Chlorophyll b
			3	Yellow	0.86	Xanthophyll
		Mancozeb	1	Grassy green	0.34	Chlorophyll a
			2	Olive green	0.16	Chlorophyll b
			3	Yellow	0.6	Xanthophyll
3	SDW control		1	Grassy green	0.08	Chlorophyll a
			2	Olive green	00	Chlorophyll b
			3	Yellow	0.71	Xanthophyll
4	Biofungicide	Neem	1	Grassy green	0.10	Chlorophyll a
			2	Olive green	0.11	Chlorophyll b
			3	Yellow	0.55	Xanthophyll
		Turmeric	1	Grassy green	00	Chlorophyll a
			2	Olive green	00	Chlorophyll b
			3	Yellow	0.52	Xanthophyll
		Cow urine	1	Grassy green	0.1	Chlorophyll a
			2	Olive green	0.12	Chlorophyll b
			3	Yellow	0.96	Xanthophyll
		T. viride	1	Grassy green	0.5	Chlorophyll a
			2	Olive green	0.14	Chlorophyll b
			3	Yellow	0.7	Xanthophyll



(A) (B)

Fig. No. 4: A. Chlorophyll extract from hybrid, control, biofungicide. B. Chlorophyll extract from fungicide.

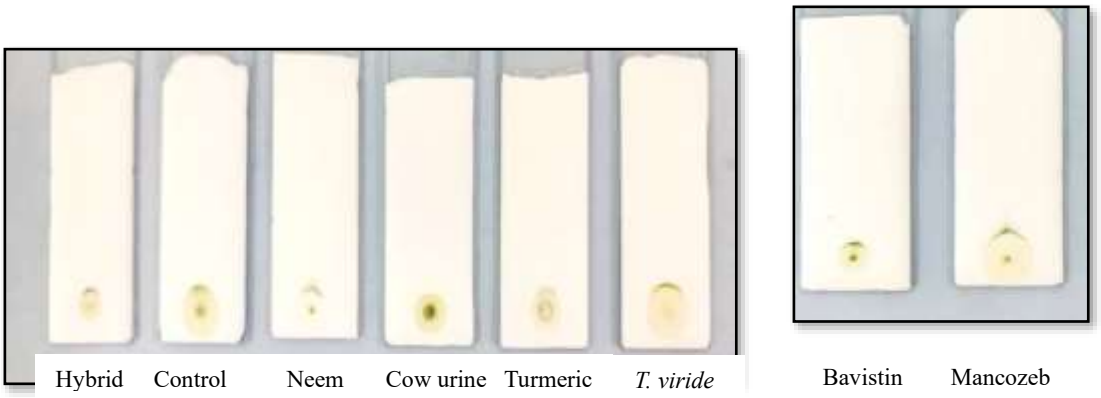


Fig. No. 5: Thin layer chromatography showing chlorophyll a, chlorophyll b and xanthophyll.

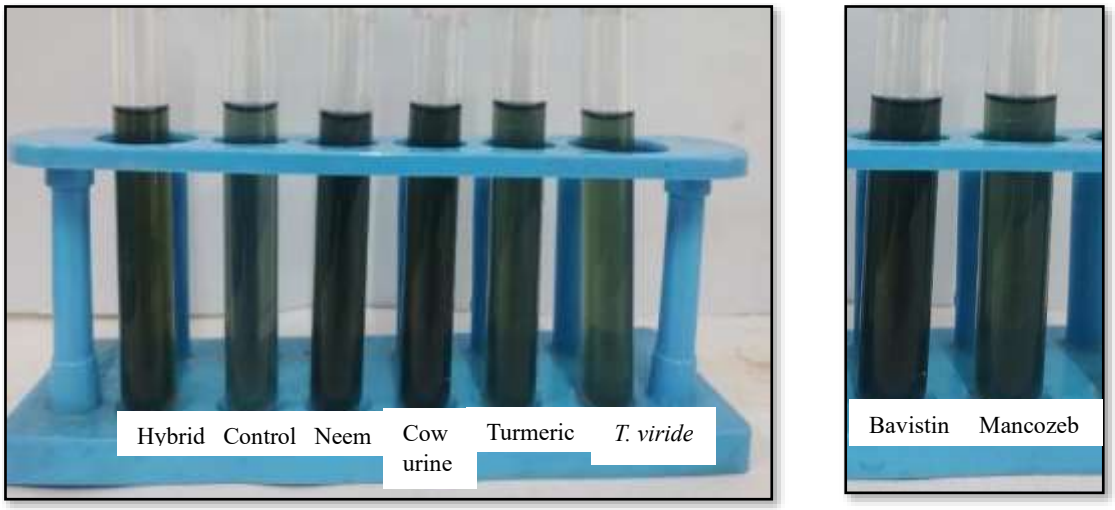


Fig. No. 6: Phenol extract from Hybrid, control, biofungicide and fungicide.

CONCLUSION

Seed-borne pathogen is associated with untreated seeds of chilly which have significantly reduced the germination of seeds. The treated seeds eliminated the seed-borne fungi improve the germination of the seed and ultimately increased the crop yield. Biofungicide is mainly used to control the activity of pathogenic fungi. Biofungicide-treated seeds germinated faster compared to fungicide and control seeds. By using fungicide and biofungicide in the plant, the growth of fungi and their spore germination was prevented. Biofungicide works best when applied preventively. It decreases the ability of pathogens to move from one plant to another plant. Hence the biofungicide treatment is the best method.

From the present investigation, it is clear that seeds-borne fungi are a threat to the health of chilly seeds. Thus, the situation demands that due attention should be paid to the health status of chilly seeds before sowing. Seed treatment may be a quick technique, applicable in this regard as it reduces or eliminates seed-borne fungi and also increases seed germination. Greenhouse studies revealed that the highest growth was recorded in hybrid seeds and protection from fungal pathogens was observed in fungicide (Bavistin and Mancozeb) treated seeds and least in control (distilled water) seeds. Among biofungicide-treated seeds *Trichoderma viride* stands best followed by Neem, cow urine, Turmeric treated seeds. By the above investigation, it is necessary to treat seeds before planting. If we continue putting our efforts only into production of crops without protecting them from seed-borne pathogens the yield will decline. So, we should practice seed treatment to achieve sustainable agricultural production. Growth promotion is offered by hybrid seeds and protection is offered by fungicide-treated seeds. Hybrid seeds or fungicides can either promote growth or offer protection respectively but the use of bio fungicide will enhance overall growth and also offer protection to chilly seeds.

DISCUSSION

Approximately 75% of the farmer surveyed in the study area reported using seeds they have saved from rotten chilli seeds for planting. This practice allows them to conserve their limited resources instead of purchasing new seeds. Over 70% of farmer in developing countries rely on their saved seeds for planting year after year. Unfortunately, none of these farmers treated their chilli seeds with suitable fungicide or bio-fungicide extract prior to planting. This continuous use of untreated, saved seeds may lead to a higher prevalence of seed born disease.

The results in respect of blotter paper method on percent associated of seeds borne fungi of different varieties of chilli seeds. Association of six fungi were observed in chilli seeds sample were collected from the different field. *Alternaria* spp, *Colletotrichum* spp, *Cladosporium* spp, *Curvularia* spp, *Cladosporium* spp, *Rhizopus* spp, were found to be associated with chilli seeds were seeds treated with different fungicide and bio-fungicide extract, control, hybrid seeds which significantly reduced germination. Highest seed borne pathogen was found to be in the control treated seeds and lessor seed borne pathogen were found to be in the Fungicide treated seeds (Bavistin and mancozeb) and hybrid seeds. Better seed born pathogen was observed in bio-fungicide treated seeds that is *Trichoderma viride* followed by neem, turmeric, cow urine treated seeds. The showed damage of seeds to varying degrees, causing seed shrinkage.

In percent seed germination, percent healthy seedling and seedling height significantly different in response to different seeds treating agents like fungicide (Bavistin and Mancozeb) and bio-fungicide (Neem, Turmeric, Cow urine, *Trichoderma viride*) hybrid seeds compared to control treated seeds. The Hybrid seeds showed percent increasing in germination 94% in root and shoot length secondly, the Fungicide (Bavistin-92%) and thirdly, Biofungicide (*Trichoderma viride*- 88%) followed by Fungicide

(Mancozeb- 80%), biofungicide (Neem- 74%, Cow urine- 70%, Turmeric- 68%) treated seeds and control-treated seeds showing the lesser in germination 64% in development of root and shoot length compare to all treatments.

The lowest vigor index was 147.62 observed in control seeds and fungicide (Bavistin and Mancozeb mg/l) was (352.17 and 308.13) and in treatment of Biofungicide (*Trichoderma viride*, neem, cow urine, turmeric mg/l) was (344.73, 227.39, 163.69, 150.35) and the highest increase vigor index was 395.34 observed in hybrid Chilly seedlings.

In the present study Biofungicide is the treatment is the best method which significantly reduced seed borne fungal pathogen of cilli, simultaneously increased the germination percentages and enhance the growth characters of chilli seedling.

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