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Evaluation of Effect of Seed Borne Nature of Chilli Anthracnose Caused by *Colletotrichum capsici* on Seed Quality Parameters

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ABSTRACT

The disease anthracnose, caused by *Colletotrichum capsici*, is a significant and destructive disease affecting chilli fruits, leading to considerable reductions in both fruit quality and seed yield. Anthracnose diminishes both the dry weight of the fruit and the quantities of capsaicin and oleoresin. By considering these criteria, a research work was conducted to evaluate the pathogenicity of seed borne chilli anthracnose disease caused by *Colletotrichum capsici*. When naturally infected seed sample, artificially inoculated seed sample, apparently healthy seed sample and surface sterilized apparently healthy seed samples were tested for seed quality parameters, per cent seed infection of mycoflora differed significantly among treatments, surface sterilized seed sample recorded least value for seed mycoflora (0.00%), highest germination percentage (81.25%), maximum shoot length (4.53 cm), root length (3.90 cm), mean seedling length (8.43 cm), mean seedling dry weight (19.54 mg), seedling vigour index-I (685) and seedling vigour index-II (1588). In contrast healthy inoculated seed sample recorded highest per cent seed infection of *Colletotrichum capsici* (66.00%). Least germination of 24.33 per cent, minimum value of shoot length (3.10 cm), root length (1.40 cm), mean seedling length (4.50 cm), mean seedling dry weight (10.00 mg), seedling vigour index-I (109) and seedling vigour index-II (243) was noticed in naturally infected seed sample. The study demonstrated the importance of seed health during seed certification of chilli seeds to ensure disease free seeds to farmers.

Keywords : *Colletotrichum capsici*, Pathogenicity, Seed infection, Seed quality parameters

AMONG the abundant wealth of variety of spices grown in India, hot-pepper (*Capsicum annuum* L.) also known as chilli, belongs to the Solanaceae family with chromosome number 2n=24. Capsicum is a genus native to the America, it was originally cultivated and domesticated in the tropical regions of the America. This spicy crop was introduced by the Portuguese in the 17th century in India (Saxena *et al*., 2016). Globally, chillies are cultivated across 1,856.64 thousand hectares, yielding approximately 4.62 million tonnes of dry fruits and 1,987.05 hectares yielding 36.09 million tonnes of fresh fruits. The average yield for dry chillies stands at approximately 2.55 tonnes per hectare, while green chillies boast a higher yield of around 18.53 tonnes per hectare (FAO, 2017).

Anthracnose, caused by *Colletotrichum capsici*, is a significant and destructive disease affecting chilli fruits, resulting in substantial losses in fruit quality and seed yield. Fungal diseases, particularly anthracnose, significantly impact the market value of fruit and seed quality, potentially resulting in yield losses of up to 50 per cent (Pakdeevaraporn *et al*., 2005).

In the dieback stage of the disease, the initial signs of infection manifest as sunken lesions on the growing tips, eventually resulting in the death of the growing tip. Subsequently, the infection progresses backward along the branch. Heavily infected fruits exhibit a straw coloration and are marked by numerous concentric rings of acervuli. Capsicum fruit rot diminishes both the dry weight of the fruit and the quantities of capsaicin and oleoresin. (Mistry *et al*., 2008). Chilli anthracnose is attributed to five species within the genus of *Colletotrichum* (Kim *et al.*, 1987). By considering this criterion, a research work was framed to evaluate the pathogenicity of seed borne chilli anthracnose disease caused by *Colletotrichum capsici*.

Material and Methods

The current study was conducted at AICRP on Seed (Crops), National Seed Project, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bengaluru, Karnataka. The fresh seeds of paprika chilli variety OLA-1 were obtained from Omni Activa Private Limited, Bengaluru. The infected chilli plant parts such as leaves, stems, twigs and fruits confirmed on the basis of typical symptoms exhibited by the pathogen were collected and pathogen was isolated. Chilli fruits from OLA-1 variety both healthy and infected were collected from Punya Koti Farm Kotiganahalli, Kolar, Karnataka, India. Healthy seeds were extracted from the healthy fruits while, infected seeds were obtained from fruits exhibiting anthracnose symptoms. Surface sterilization of seeds was done by dipping them into 1 per cent sodium hypochlorite solution followed by adequate rinsing (2-3 times) in distilled water and were kept on sterilized blotter paper for drying.

Pathogenicity Test

For inoculation, pinprick method on chilli fruit developed by Naik and Rawal (2002) was followed. Ten days old cultures were used for artificial inoculation. Chilli fruits harvested at red ripened stage were surface sterilized with one per cent sodium hypochlorite solution and then washed in two changes of sterile water. Thereafter, the fruits were pricked with pin bundles specially designed for pricking. The pinpricked fruits were then dipped in spore suspension having 1 X 10^6 spores /ml for one minute. Further these fruits were kept for incubation on a perforated tray under humid chamber. The humid chamber was prepared by keeping water in the tray, which was placed below the perforated tray kept with inoculated fruits. Three wet cotton pieces were placed on the tray. The tray was covered with polythene sheet to maintain the relative humidity of over 90 per cent and then incubated at $25^o \pm 1^oC$ for eight days. After the development of symptom on the chilli fruits, reisolation of the fungus was made from the affected portion of the fruit as per the method described earlier and Koch's postulates were proved. Then the seeds were extracted from fruits and the pathogen was reisolated as per the recommended procedure (Fig. 1).

Reisolation of Pathogen on Potato Dextrose Agar

Infected seeds extracted from artificially inoculated fruits were placed on the sterilized potato dextrose agar medium (20 ml) was poured aseptically in to sterilized petri plates of 90 mm diameter. After solidification, medium and plates were incubated at 25 ± 1°C. Same seeds were observed for the presence of fungal growth commencing from 4^{th} day of incubation (Fig. 1 and 2).

Seed to Seedling Transmission

Top of paper method for transmission studies. Transmission of the pathogen from the infected seeds to seedlings was studied through top of paper method (Yago *et al*., 2011). Randomly selected 100 seeds were placed, ten seeds per petriplate on two layers of moist butter paper. These petriplates were incubated in seed germinator at 25 ± 1°C for 14 days. After 10 days of incubation seedlings symptoms were observed under stereo microscope.

Per Cent Seed Infection

Detection and identification of per cent seed infection was done by using blotter paper method. Twenty-five seeds from three replications of each treatment were placed equidistantly in sterile glass petri plates with two moist blotter papers (Whatman No. 1) and incubated at 25°C for 8 days in the light. Then, the seeds were examined for the presence of seed

a) Inoculation by pin prick method and incubation in moist chamber

b) *In-vitro* development of symptoms on fruits

c) Mycelial growth on chilli fruits

d) Artificially inoculated seeds tested on PDA

Fig. 1 : Proving pathogenicity of *Colletotrichum capsici*

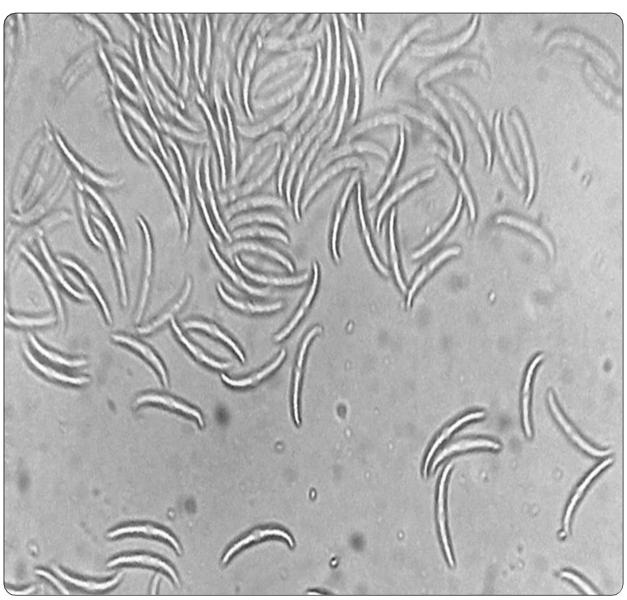


Fig. 2 : Spores of *Colletotrichum capsici* under microscope

pathogens were identified and results were expressed as in the per cent infection.

Statistical Analysis

The experiment was laid out in Completely Randomized Design (CRD) with three replications and the data obtained was analyzed by using Analysis of Variance (ANOVA) technique. Standard error of difference was calculated for each treatment effect and critical difference (CD) was calculated at 1 per cent probability level to compare the mean difference among the treatments for lab conditions and critical difference (CD) was calculated at 5 per cent probability level to compare the mean difference among the treatments in green house conditions (Gomez and Gomez, 1984). Other statistical analysis *viz*., calculation of correlation coefficients was done using MS-excel.

Results and Discussion

Effect of Pathogenicity of *Colletotrichum capsici* on Seed Quality Parameters

When naturally infected seed sample, artificially inoculated seed sample, apparently healthy seed sample and surface sterilized apparently healthy seed samples were tested for seed quality parameters, per cent seed infection of mycoflora differed significantly among treatments. Surface sterilized seed sample recorded least value for seed mycoflora (0.00%), highest germination percentage (81.25%), maximum shoot length (4.53 cm), root length (3.90 cm), mean seedling length (8.43 cm), mean seedling dry weight (19.54 mg), seedling vigour index-I of 685 and seedling vigour index-II of 1588. In contrast healthy inoculated seed sample recorded highest per cent seed infection of *Colletotrichum capsici* (66.00%). Least germination of 24.33 per cent, minimum value of shoot length (3.10 cm), root length (1.40 cm), mean seedling length (4.50 cm), mean seedling dry weight (10.00 mg), seedling vigour index-I (109) and seedling vigour index-II (243) was noticed in naturally infected seed sample (Table 1 and 2). Such findings were also observed by Nagarjuna (2019) in chilli seeds, who reported a 40 per cent reduction in germination of naturally infected seeds compared to healthy seeds. Fungal pathogens kill plant seeds before seed germination (Li *et al*., 2019 and Roopashree & Rajendra Prasad, 2021). Fungi feeds

Table 1

Effect of pathogenicity of *Colletotrichum capsici* on seed quality parameters

Treatment	Per cent seed infection	Seed germination (%)	Shoot length (cm)	Root length (cm)
T_1: Naturally infected seed sample	42.33	24.33	3.10	1.40
T_2: Artificially inoculated seed sample	66.00	53.50	3.20	2.00
T_3: Apparently healthy seed sample	1.00	78.33	4.50	3.50
T_4: Surface sterilized apparently healthy seed sample	0.00	81.25	4.53	3.90
Mean	27.33	59.35	3.83	2.70
S. Em±	0.677	0.703	0.070	0.050
CD (P=0.01)	2.924	3.039	0.304	0.216
CV (%)	4.954	2.370	3.670	3.704

Table 2

Effect of pathogenicity of *Colletotrichum capsici* on seedling vigour parameters

Treatment	Mean seedling length (cm)	Mean seedling dry weight (mg)	Seedling vigour index I	Seedling vigour index II
T_1: Naturally infected seed sample	4.50	10.00	109	243
T_2: Artificially inoculated seed sample	5.20	15.50	278	830
T_3: Apparently healthy seed sample	8.00	19.50	627	1527
T_4: Surface sterilized apparently healthy seed sample	8.43	19.54	685	1588
Mean	6.53	16.13	425	1047
S. Em±	0.087	0.301	8.726	24.168
CD (P=0.01)	0.376	1.300	37.695	104.402
CV (%)	2.664	3.730	4.109	4.616

on their substrates, including seeds releasing lytic enzymes involved in substrate breakdown (Griffin, 1966). Fungi damage seeds by releasing toxins that may inhibit germination, cause membrane damage and/or increase solute leakage from seeds (Wagner and Mitschunas, 2008). The infection disrupts the seed's ability to effectively utilize nutrients, further restricting shoot and root development. Additionally, infected seeds may experience heightened stress, which can negatively impact their physiological functions and hinder normal growth processes (Pérez *et al*., 2023).

Correlation between Per cent Seed Infection and Various Seed Quality Parameters

Per cent seed infection is negatively correlated with all other parameters. The correlation studies were conducted to study the pre cent seed infection on seed quality parameters namely seed germination, shoot length, root length, mean seedling length, mean seedling dry weight and seedling vigour index I and II. The results clearly revealed that there is no positive correlation between seed infection and quality parameters. The highest negative correlations are with shoot length (r = -0.93797), root length (r = -0.86551), mean seedling length (r = -0.89862), seedling vigour index I (r = -0.8477) and seed germination (r = -0.71793). This suggests that as seed infection increases, these parameters significantly decrease. Seed germination shows a strong positive correlation with most parameters. It has a high positive correlation with root length (r = 0.962905), mean seedling length (r = 0.948112), seedling vigour index I (r = 0.97637) and seedling vigour index II (r = 0.996693). This indicates that higher germination rates are associated with longer root lengths and higher seedling vigour. Seedling vigour index I has very strong correlations with root length (r = 0.99771) and mean seedling length (r = 0.994339). Seedling vigour index II is highly correlated with seed germination, root length and mean seedling dry weight. It has negative correlations with per cent seed infection (r = -0.77207) (Table 3 and Fig.3).

The data highlights a clear negative relationship between seed infection and seedling health. The strong negative correlations between per cent seed infection and critical growth parameters such as seedling shoot length, root length and mean seedling length demonstrate that higher infection rates significantly hinder seedling development. This is in consistent with the fact that *Colletotrichum capsici* infection in chilli seeds can lead to poor seedling vigour, reduced growth. Seeds with higher germination rates tend to produce healthier, more vigorous seedlings with longer roots and shoots, which are key indicators of seedling quality and future plant performance (Kumudkumar *et al*., 2004 and Seema *et al*., 2023).

TABLE 3

Correlation between per cent seed infection and various seed quality parameters

Correlation Coefficients	Per cent seed infection	Seed germination (%)	Shoot length (cm)	Root length (cm)	Mean seedling length (cm)	Mean seedling dry weight (mg)	Seedling vigour index I	Seedling vigour index II
Per cent seed infection	1.000							
Seed germination (%)	-0.71793	1.000						
Shoot length (cm)	-0.93797	0.914543	1.000					
Root length (cm)	-0.86551	0.962905	0.980348	1.000				
Mean seedling length (cm)	-0.89862	0.948112	0.992866	0.996877	1.000			
Mean seedling dry weight (mg)	-0.67946	0.99768	0.89166	0.943113	0.943113	1.000		
Seedling vigour index I	-0.8477	0.97637	0.977467	0.99771	0.994339	0.960919	1.000	
Seedling vigour index II	-0.77207	0.996693	0.944388	0.979686	0.970203	0.990238	0.990089	1.000

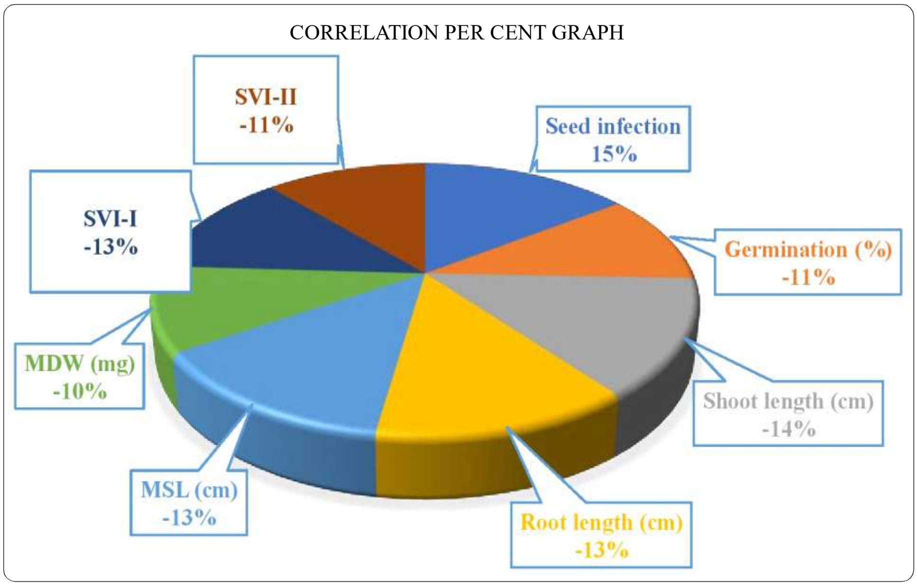


Fig. 3 : Correlation between per cent seed infection and seed quality parameters

Effect of Pathogenicity of *Colletotrichum capsici* on Seedling Emergence Paraments in Chilli

Naturally infected seed sample, artificially inoculated seed sample, apparently healthy seed sample and surface sterilized apparently healthy seed sample were used for seed to plant transmission study. This study was conducted under controlled conditions in polyhouse (Fig. 4). The result of the experiment (Table 4) indicated that surface sterilized apparently healthy seed sample showed significantly higher per cent of portray emergence of 78.50 per cent and it was followed by apparently healthy seed sample (76.00%). Naturally infected seed sample showed significantly lower per cent of portray emergence of 20.75 per cent. Significantly lower seedling mortality of 21.50 per cent was noticed in surface sterilized apparently healthy seed sample followed by 24.00 per cent seedling mortality in apparently healthy seed sample. Naturally infected seed sample showed significantly highest seedling mortality (79.25%). Seedling length, 30 days after sowing showed significant results among treatments, surface sterilized apparently healthy seed sample (10.5 cm) recorded maximum seedling length followed by apparently healthy seed sample (9.58 cm). The least value for seedling length (5.25 cm) was recorded in naturally infected seed sample (Table 4). The research outcomes are in corollary with research findings of Kumudkumar *et al*. (2004) in chilli.

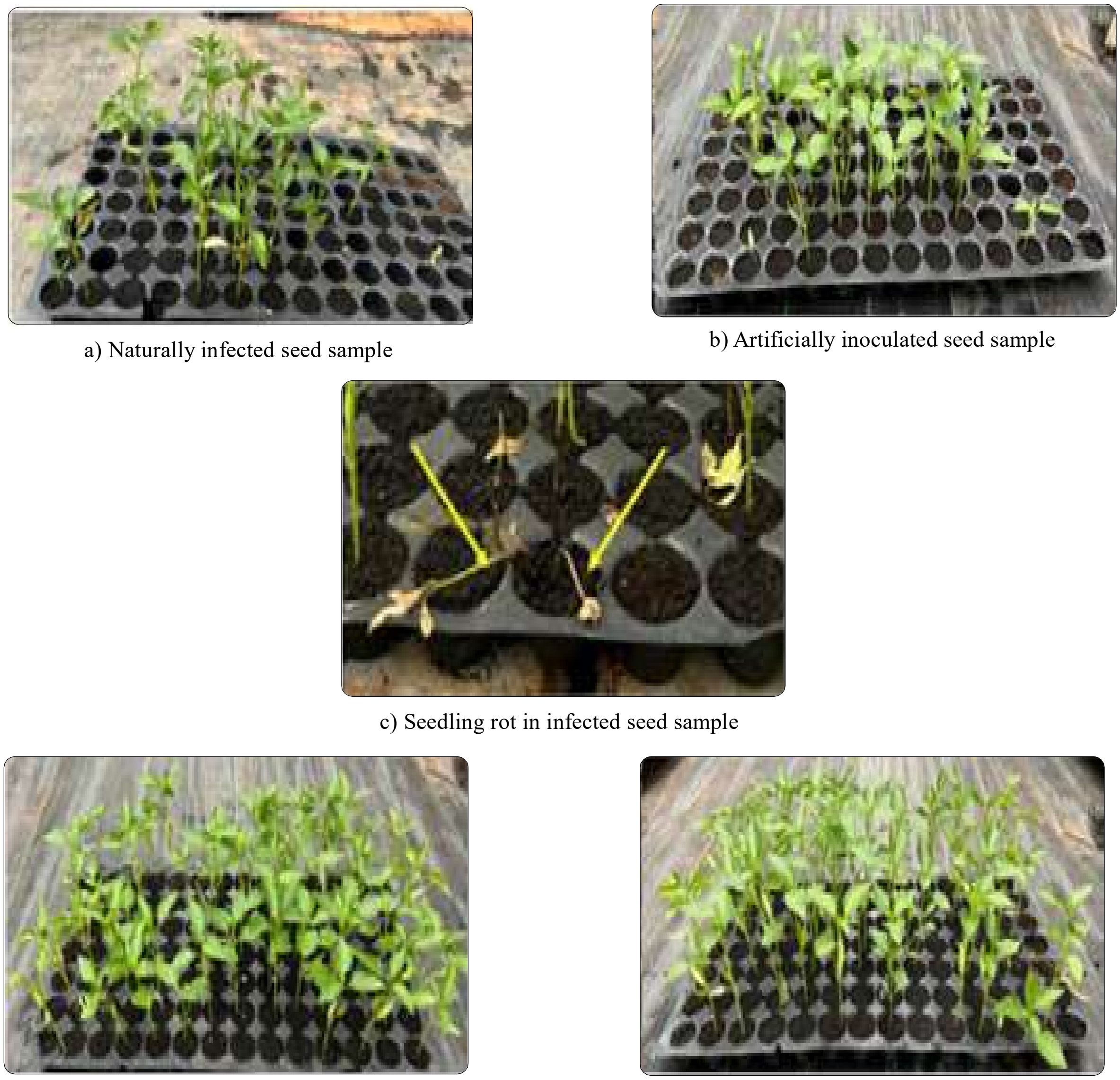
TABLE 4

Effect of pathogenicity on seedling survival and mortality per cent

Treatment	Mortality (%)	Seedling length (cm) (30 DAS)
T_1: Naturally infected seed sample	79.25	5.25
T_2: Artificially inoculated seed sample	52.75	7.00
T_3: Apparently healthy seed sample	24.00	9.58
T_4: Surface sterilized apparently healthy seed sample	21.50	10.5
Mean	44.38	8.08
S. Em±	0.743	0.200
CD (P=0.05)	2.289	0.616
CV (%)	3.349	4.947

DAS: Days after sowing

The pot culture studies aimed at investigating the transmission of *Colletotrichum capsici* from seeds to plants showed that both naturally infected and artificially inoculated seeds experienced a significant



a) Naturally infected seed sample

b) Artificially inoculated seed sample

c) Seedling rot in infected seed sample

d) Apparently healthy seed sample

e) Surface sterilized apparently healthy seed sample

Fig. 4 : Effect of pathogenicity of *Colletotrichum capsici* on seedling growth

reduction in germination rates and increased seedling mortality within 15 days after sowing. The reduction in plant height in chilli plants infected with *Colletotrichum capsici* at all stages of growth is due to tissue damage, impaired nutrient and water uptake, decreased photosynthesis and general stress-induced growth limitations (Than *et al*., 2008). This study highlights the critical impact of seed-borne infections. Supporting this, Kumudkumar *et al*. (2004) found that *Colletotrichum capsici* was transmitted to young seedlings through inoculum from infected seeds *via* local contact. They also observed that heavily infected seed samples exhibited poor germination rates and high mortality of seedlings decreased photosynthesis and general stress-induced growth limitations (Than *et al*., 2008). This study highlights the critical impact of seed-borne infections. Supporting this, Kumudkumar *et al*. (2004) found that *Colletotrichum capsici* was transmitted to young seedlings through inoculum from infected seeds *via* local contact. They also observed that heavily infected seed samples exhibited poor germination rates and high mortality of seedlings.

The study demonstrates the importance of seed health and seed certification of chilli seeds for proving farmers disease free seeds. Surface sterilized seed sample recorded least value for seed mycoflora (0.00%), highest germination percentage (81.25%), maximum shoot length (4.53 cm), root length (3.90 cm), mean seedling length (8.43 cm), mean seedling dry weight (19.54 mg), seedling vigour index-I (685) and seedling vigour index-II (1588). Least germination of 24.33 per cent, minimum value of shoot length (3.10 cm), root length (1.40 cm), mean seedling length (4.50 cm), mean seedling dry weight (10.00 mg), seedling vigour index-I (109) and seedling vigour index-II (243) was noticed in naturally infected seed sample. Correlation studies showed that the highest negative correlations are with shoot length (r = -0.93797) and root length (r = -0.86551). Whereas, higher per cent of portray emergence (78.50%) and least seedling mortality (21.50%) was recorded in surface sterilized seed sample. Naturally infected seed sample showed significantly lower per cent of portray emergence (20.75%) and highest seedling mortality (79.25%). Additionally, statistically significant result was obtained for seedling length at 30 DAS, surface sterilized apparently healthy seed sample (10.5cm) recorded maximum seedling length and least value was recorded in naturally infected seed sample (5.25 cm). Therefore, early seed health detection methods and proper seed treatment before sowing would aid in controlling the yield loses caused by *Colletotrichum capsici* in chilli.

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