EFFECT OF *FUSARIUM* CULTURE FILTRATE (C.F.) ON SEED GERMINATION, ROOT-SHOOT LENGTH AND SEED VIGOR

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Abstract

Fusarium species are important phytopathogens known to produce an array of toxic metabolites that adversely affect seed germination and early seedling growth. The present study investigates the effect of different *Fusarium* species culture filtrate on seed germination, root–shoot length and Seed Vigor in the selected test crop i.e. Chickpea, Soyabean and Pigeon pea. The culture filtrates of *Fusarium* spp. were prepared from actively growing *Fusarium* cultures and applied to seeds under controlled laboratory conditions. Seeds treated with culture filtrate exhibited a significant decline in germination percentage compared to the control. Moreover, marked reductions in both root and shoot lengths were observed, indicating the strong inhibitory potential of metabolites released by *Fusarium* species. The severity of inhibition increased with increasing concentrations of the culture filtrate, confirming a dose-dependent phytotoxic response. These findings highlight the detrimental impact of *Fusarium*-derived metabolites on early plant growth and underscore their role in disease development and seedling mortality. The study provides useful insights for understanding host–pathogen interactions and for developing effective management strategies against *Fusarium*-induced seed and seedling infections.

Keywords: *Fusarium*, Culture Filtrate (C.F.), Seed Germination, Root-shoot Length, Seed Vigor

I. Introduction

Fusarium species represent one of the most destructive groups of soil-borne and seed-borne plant pathogens and are recognized globally for their ability to infect a wide range of economically important agricultural crops. These pathogens are responsible for several devastating plant diseases such as vascular wilt, root rot, stem rot, damping-off and leaf blight, which collectively result in substantial yield losses and reduced crop quality. The pathogenic success of *Fusarium* spp. is strongly linked to their capacity to produce a wide spectrum of secondary metabolites and toxic compounds, commonly referred to as mycotoxins. Notable among these are fusaric acid, trichothecenes, fumonisins, moniliformin, and zearalenone. These metabolites play a central role in disease development by disrupting cellular metabolic pathways, inhibiting key enzymatic processes, altering membrane permeability, and ultimately damaging host tissues. As highlighted by Desjardins (2006), these toxins not only contribute to virulence but also function as major determinants of host

specificity and symptom expression. A significant impact of *Fusarium* toxins is observed during the early developmental stages of plants, particularly at the seed germination phase. When seeds are exposed to *Fusarium* culture filtrates or purified toxins, they frequently exhibit a considerable decline in germination percentage. This reduction is primarily caused by the inhibition of early metabolic activities essential for radicle protrusion and plumule emergence. According to Nelson et al. (1993), these toxins interfere with the mobilization of stored nutrients and disrupt hormonal signaling pathways, thereby impeding the orderly progression of germination. Moreover, root and shoot development in emerging seedlings is severely hampered. Roots tend to show pronounced sensitivity to *Fusarium* toxins, often manifesting symptoms such as reduced cell division, diminished elongation, browning of tissues, and necrosis of the root tips. These alterations hinder the plant's ability to absorb water and nutrients efficiently, leading to poor seedling vigor, stunted growth, and delayed establishment (Agrios, 2005). As seedlings continue to grow in the presence of *Fusarium* toxins, further developmental abnormalities become evident. The phytotoxic metabolites induce chlorosis, reduced biomass accumulation, impaired photosynthetic efficiency, and heightened oxidative stress. Fusaric acid in particular has been associated with the production of reactive oxygen species (ROS), which damage cellular structures such as membranes, proteins, and nucleic acids. Bacon et al. (1996) reported that such toxin-induced oxidative stress results in wilting, suppressed seedling vitality, and ultimately poor plant establishment. The production of trichothecene toxins is particularly associated with the inhibition of protein synthesis in plant cells, leading to cell death, chlorotic patches, tissue collapse, and eventual necrosis (Proctor et al., 1995). These foliar symptoms not only reflect toxin activity but also serve as diagnostic markers of *Fusarium*-induced pathogenesis in natural field conditions. Comprehensive understanding of the phytotoxic effects exerted by *Fusarium* toxins on seed germination and root–shoot elongation, is crucial for devising effective disease-management strategies. Investigating the inhibitory effects of *Fusarium* culture filtrates provides valuable insight into the roles of individual metabolites in disease initiation and progression. Such knowledge is essential for identifying resistant plant genotypes, improving screening techniques, and designing integrated management approaches involving biological control agents, cultural practices, and selective fungicides. Ultimately, advancements in understanding *Fusarium* toxin-mediated interactions will contribute significantly to mitigating crop losses and promoting sustainable agricultural production.

II. Materials and Methods

1. Isolation of *Fusarium* Pathogen

Wilt-infected plants were collected from various localities of the Marathwada region, including Beed, Chhatrapati Sambhajinagar, Dharashiv, Jalna, Nanded, and Parbhani. Stem sections showing typical wilt symptoms were carefully excised and surface-sterilized using 1% mercuric chloride ($HgCl_2$) for 2 minutes. The sections were then rinsed twice with sterile distilled water to remove traces of the disinfectant and air-dried under aseptic conditions in a laminar flow cabinet. *Fusarium* isolation was performed using Potato Dextrose Agar (PDA) medium. The inoculated plates were incubated at 30 °C for 5–7 days to allow fungal growth. Emerging *Fusarium* colonies were purified and maintained through sub-culturing using the single-spore isolation technique as described by Leslie and Summerell (2006).

2. Microbes Used in the Test

The Pathogenic *Fusarium* species used in this study were isolated from wilt-infected crop plants collected from the field. The identified pathogenic isolates included *Fusarium annulatum, F. chlamydosporum, F. concentricum, F. equiseti, F. foetens, F. fujikuroi, F. hainanense, F. incarnatum, F. pernambucanum, and F. pseudocircinatum*

3. Selection of Media

During investigation Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) medium were used for the isolation and maintenance of pure cultures. The composition of the medium is as follows

i. Potato Dextrose Agar (PDA)

Peeled Potato – 200g, Dextrose – 20g, Agar-Agar – 20g, Streptomycin – 0.2g, Distilled Water (D/W) – 1000ml, pH - 5.5 to 5.6.

ii. Potato Dextrose Broth (PDB)

Peeled Potato – 200g, Dextrose – 20g, Distilled Water (D/W) – 1000ml pH - 5.5 to 5.6

4. Prepared *Fusarium* Culture Filtrate (C.F.)

The *Fusarium* culture filtrate was prepared by inoculating actively growing *Fusarium* mycelium into 100 mL of Potato Dextrose Broth (PDB) medium. After inoculation, the flasks were incubated at 30°C for 7 days under static conditions to promote optimal mycelial growth and the secretion of extracellular metabolites. At the end of the incubation period, the fungal cultures were carefully filtered through Whatman No. 1 filter paper to separate the mycelial biomass from the liquid phase. The resulting clear and transparent culture filtrate was collected in a sterile conical flask and stored appropriately for subsequent experimental analysis.

5. Effect of Culture Filtrate (C.F.) on Seed Germination and Root-Shoot Length

Seeds of three legume chickpea, soybean and pigeon pea were collected from the local market for the experiment. All seeds were surface sterilized using 1% mercuric chloride ($HgCl_2$) for 1 minute, followed by three rinses with sterile distilled water to remove any chemical residue. The sterilized seeds were then pre-soaked in *Fusarium* culture filtrate (C.F.) for 1 hour, while seeds soaked in sterile distilled water served as the control. After the pre-soaking period, the seeds were removed from the culture filtrate and rinsed again with sterile distilled water. The treated and control seeds were placed in sterile Petri plates lined with two layers of moist blotter paper and incubated at room temperature. Seed germination was monitored after 48 and 120 hours, and further observations were recorded at intervals of 3, 5, and 7 days. To determine the effect of *Fusarium* culture filtrate on root and shoot elongation of germinating seeds, the method described by Luke and Wheeler (1955) was followed. After 7 days of incubation, the lengths of roots and shoots were measured using a thread and a scale, and the results were compared with those of the control group. Germination percentage and vigour index were calculated using the standard formulas described by Suthar et al. (2014).

$$\textbf{\textit{Germination}}\ \% = \frac{\text{Total No. of Germinated Seed}}{\text{Total No. of Seeds Sown}} X\ 100$$

Vigour Index = Root Length (cm) + Shoot Length (cm) x Germination Percentage (%)

III. Result and Discussion

1. Effect of *Fusarium* Culture Filtrate (C.F.) on Seed Germination (%)

Table 1 represents the effect of *Fusarium* culture filtrate (C.F.) on the seed germination of three legume crops—chickpea, soybean, and pigeon pea, the germination was observed at 3, 5, and 7 days. The table are comparing 10 different *Fusarium* species culture filtrate treatments as compare to untreated control. Overall, the control shows the highest germination percentage across all crops and time points, indicating normal, unhindered seed germination (90–100%). Most *Fusarium* species significantly reduce germination, though the severity varies. For chickpea, several species such as *Fusarium annulatum*, *F. chlamydosporum, F. fujikuroi*, and *F. hainanense* completely inhibit germination (0%) at all intervals, highlighting their strong inhibitory effects. In contrast, *F. equiseti, F. foetens, F. incarnatum, F. pernambucanum*, and *F. concentricum* allow moderate germination (10–70%), with *F. pernambucanum* reaching as high as 70% by day 7. In soybean, inhibitory effects are milder compared to chickpea. Germination ranges from 10–70% depending on the species and duration. Species like *F. equiseti* show relatively higher germination (up to 70%), while *F. fujikuroi* and *F. hainanense* maintain low levels (10-20%). Pigeon pea shows varied responses: some *Fusarium* species, especially *F. fujikuroi*, show extreme inhibition with almost no germination. Others like *F. foetens* and *F. incarnatum* allow moderate to high germination (50–90%) by day 7. Overall, the data clearly indicate that different *Fusarium* species have different levels of phytotoxicity, with some causing complete inhibition of seed germination and others allowing partial growth. The control consistently shows the highest germination, confirming that observed reductions are due to the effect of the culture filtrates (Figure-01,02 &03).

2. Effect of *Fusarium* Culture Filtrate (C.F.) on Root-Shoot Length (cm) and Seed Vigor

The table 2 represents the effect of *Fusarium* culture filtrates (C.F.) on the root length, shoot length, and vigor index of three legume crops—chickpea, soybean, and pigeon pea. The table are comparing 10 different *Fusarium* species culture filtrate treatments as compare to untreated control Overall, the control group recorded the highest growth parameters across all three crops, confirming normal, uninhibited growth in the absence of fungal metabolites. Among the treatments, different *Fusarium* species exhibited varied levels of toxicity, significantly reducing root and shoot development. In chickpea, only a few species such as *Fusarium equiseti* (0.64 cm root, 0.22 cm shoot, vigor index 11.64) and *F. pernambucanum* (2.06 cm root, 0.62 cm shoot, vigor index 46) showed measurable growth, while many species caused complete inhibition (0.00 values) i.e. *Fusarium annulatum*, *Fusarium chlamydosporum, Fusarium fujikuroi,* and *Fusarium hainanense*. In soybean, *F. equiseti* caused the highest vigor index (612.64), even though root and shoot lengths were moderately suppressed, whereas *F. incarnatum* also recorded considerably high vigor (270.46). In pigeon pea, *F. equiseti* again showed a strong inhibitory or stimulatory effect with a vigor index of 207.91, while *F. incarnatum (*161.43) and *F. foetens* (86.38) also induced notable responses. Some species such as *F. annulatum, F. fujikuroi,* and *F. hainanense* caused severe suppression in almost all crops, reflected in extremely low or zero growth values. Overall, the table highlights that *Fusarium* culture filtrates influence seedling growth differently depending on the species, with certain filtrates showing strong inhibitory effects while a few demonstrate comparatively less toxicity.

Table 1: Effect of *Fusarium* Culture Filtrate (C.F.) on Seed Germination (%)

Sr. No.	Name of *Fusarium* Species	Chickpea			Soyabean			Pigeon Pea		
		3 Day	5 Day	7 Day	3 Day	5 Day	7 Day	3 Day	5 Day	7 Day
1	*Fusarium annulatum*	00	00	00	10	10	10	10	40	60
2	*Fusarium chlamydosporum*	00	00	00	20	20	20	10	30	30
3	*Fusarium equiseti*	40	50	50	40	50	70	50	70	70
4	*Fusarium fujikuroi*	00	00	00	10	10	20	00	00	00
5	*Fusarium foetens*	40	50	50	30	40	40	70	80	90
6	*Fusarium hainanense*	00	00	00	00	10	10	60	60	70
7	*Fusarium incarnatum*	30	30	40	40	40	50	60	70	80
8	*Fusarium pernambucanum*	40	60	70	20	20	20	00	20	20
9	*Fusarium pseudocircinatum*	00	20	20	10	20	30	30	50	50
10	*Fusarium concentricum*	10	10	10	30	50	50	50	50	50
11	Control	90	100	100	90	100	100	90	100	100

Table 2: Effect of *Fusarium* Culture Filtrate (C.F.) on Root-Shoot Length (cm) and Vigor Index

Sr. No.	Name of *Fusarium* Species	Chickpea			Soyabean			Pigeon Pea		
		Root Length	Shoot Length	Vigor Index	Root Length	Shoot Length	Vigor Index	Root Length	Shoot Length	Vigor Index
1	*Fusarium annulatum*	00	00	00	0.24	00	0.24	0.56	00	0.56
2	*Fusarium chlamydosporum*	00	00	00	3.03	1.84	40.1	0.42	0.30	9.42
3	*Fusarium equiseti*	0.64	0.22	11.64	3.64	8.07	612.64	1.41	2.95	207.91
4	*Fusarium fujikuroi*	00	00	00	0.77	00	0.77	00	00	00
5	*Fusarium foetens*	0.72	0.85	43.22	1.06	2.95	119.6	0.88	0.95	86.38
6	*Fusarium hainanense*	00	00	00	0.3	00	0.3	0.44	1.31	92.14
7	*Fusarium incarnatum*	0.35	0.30	12.35	2.96	5.35	270.46	1.43	2.0	161.43
8	*Fusarium pernambucanum*	2.06	0.62	46	1.4	2.67	54.8	0.15	00	0.15

9	*Fusarium pseudocircinatum*	0.15	00	0.15	1.45	2.33	71.3	0.44	0.54	27.44
10	*Fusarium concentricum*	0.51	0.21	2.61	3.34	6.2	313..4	0.81	2.25	113.31
11	Control	7.2	3.57	364.02	10..04	11.87	1191.04	3.25	5.27	530.25

IV. Conclusion

The effect of different *Fusarium* culture filtrate (C.F.) on seed Germination, Root-Shoot Length and Seed Vigor demonstrates that the metabolites produced by the *Fusarium* pathogen significantly inhibit early plant development. Seeds treated with the *Fusarium* culture filtrate showed reduced Seed Germination percentage, shorter root and shoot lengths, and overall weaker seedling vigor compared to the untreated control. This indicates the phytotoxic nature of *Fusarium* metabolites, which adversely affect cellular processes essential for Germination and Growth. The findings highlight the role of pathogenic *Fusarium* secretions in suppressing crop establishment and emphasize the need for effective disease-management strategies to protect seeds and young seedlings from *Fusarium*-induced stress.

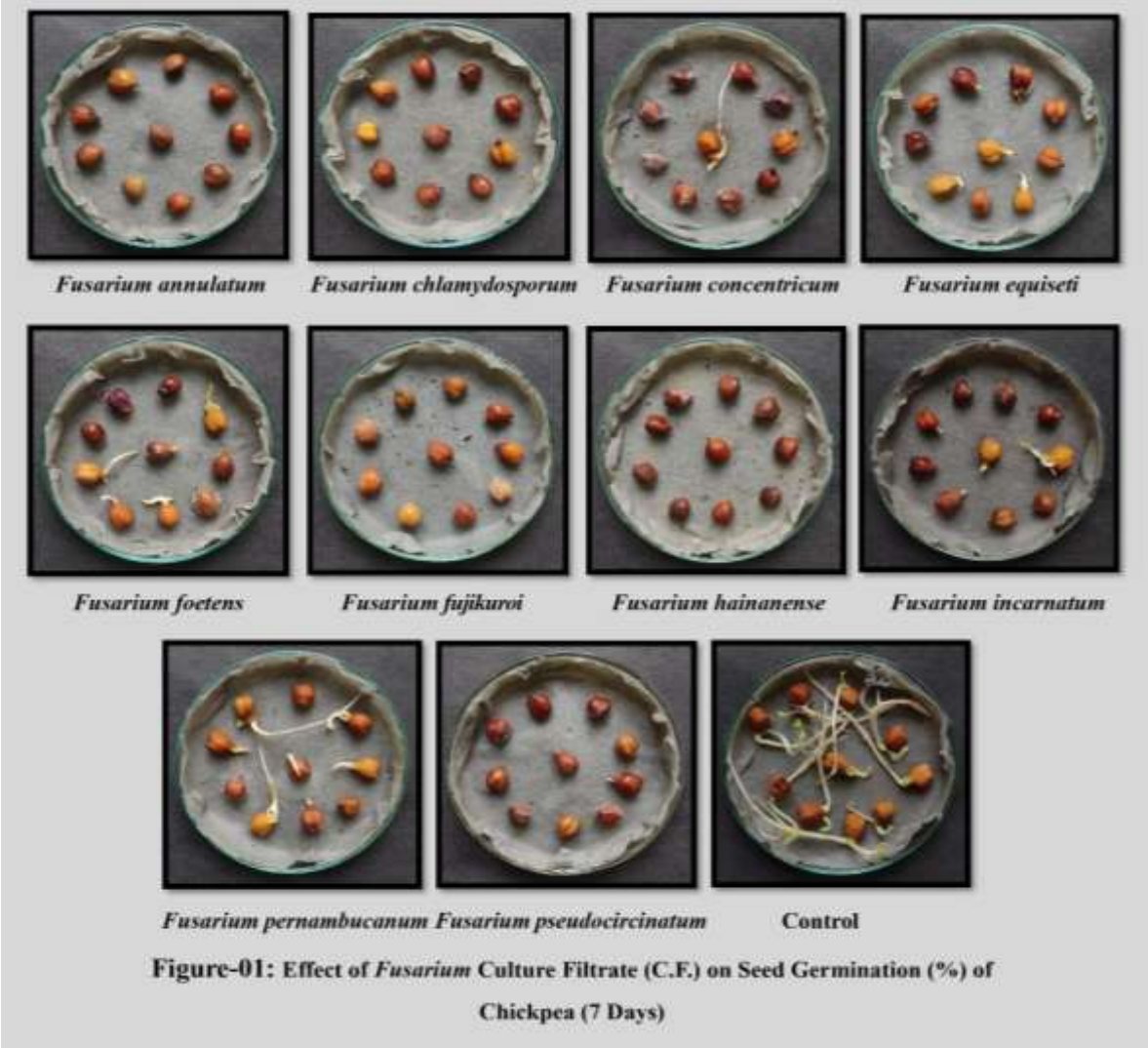

Figure-01: Effect of *Fusarium* Culture Filtrate (C.F.) on Seed Germination (%) of Chickpea (7 Days)

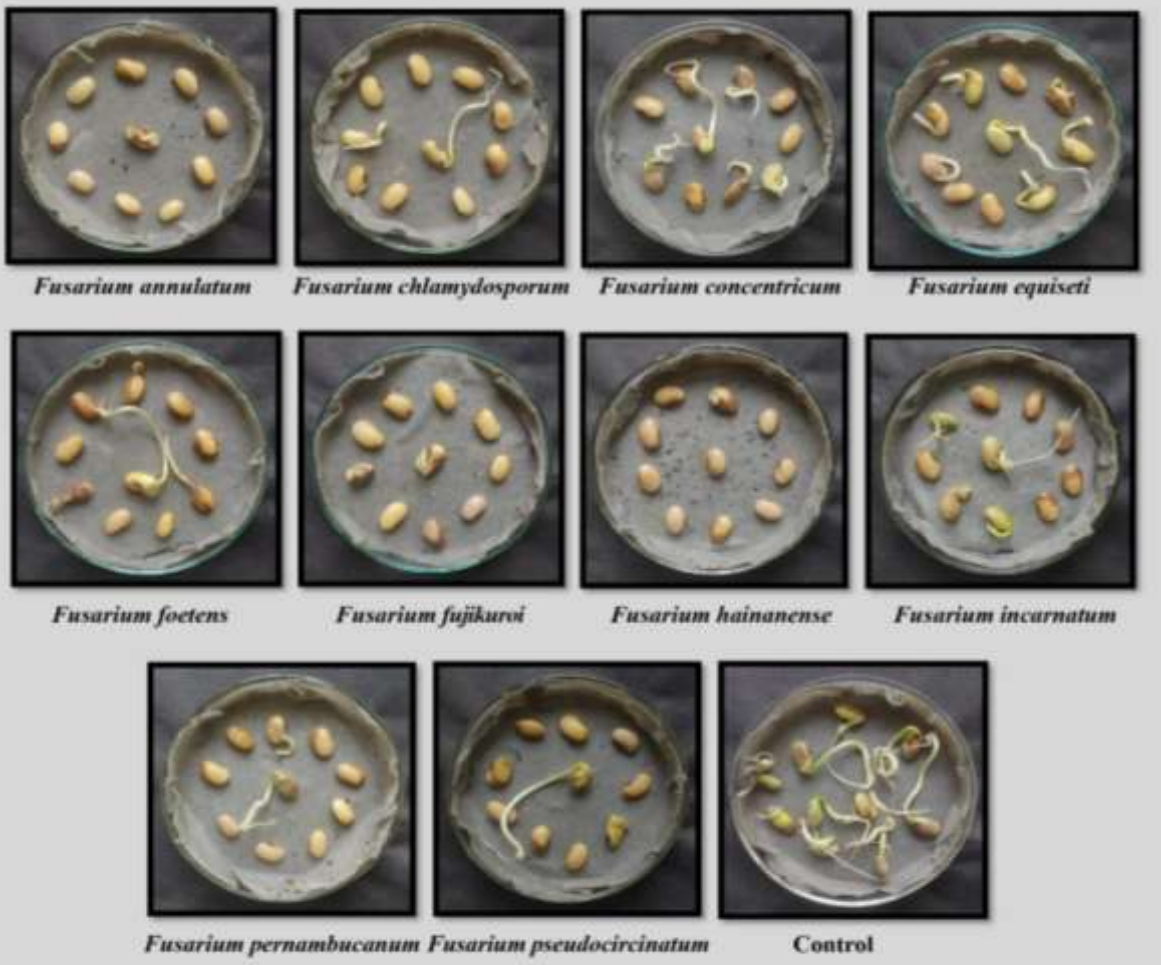

Figure-02: Effect of *Fusarium* Culture Filtrate (C.F.) on Seed Germination (%) of

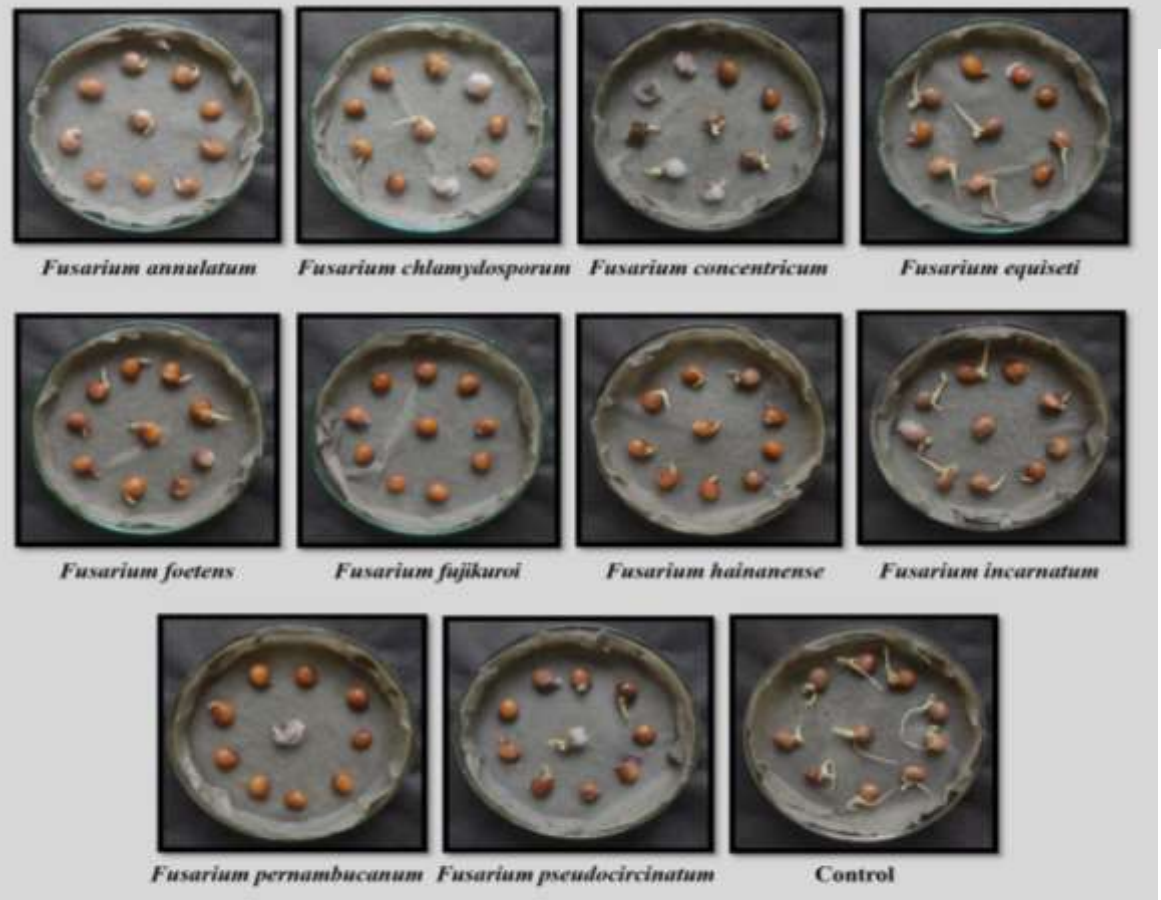

Figure-03: Effect of *Fusarium* Culture Filtrate (C.F.) on Seed Germination (%) of Pigeon Pea (7 Days)

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