Detection of Seed Borne Fungi Associated with *Glycine max* and its Management Through Physio–Chemical Treatments

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Abstract

Soybean (*Glycine max*) is an annual oilseed crop that is mostly farmed for its edible seeds. It offers 40% of the most affordable source of protein for animal and human feed. The crop suffers from various diseases, reducing its yield either slightly or even eliminating it. The main hindrance to soybean production is the fungus that lives in the seeds. The present study aimed to evaluate the impact of various seed treatments on the control of soybean seed-borne fungus infections. This study used treatments, namely physical therapy by heating the seeds in a microwave at a temperature of 40^{o}C for 10, 20 and 30 seconds and chemical treatment by soaking the seeds in a fungicide with active ingredient difenoconazole with a concentration of 1%, 2% and 3%. Seeds without heating and as control are soaking fungicides. Each treatment was repeated three times. The seeds that have been given treatments are then planted using the growing test technique and incubated for seven days. Furthermore, the seed viability and the growth of pathogenic fungi were observed at the end of incubation. The results showed that soybean seed germination was not affected by physical and chemical treatments. The seed viability of 100% with or without treatment. This was confirmed by the findings of seed-borne fungi (*Fusarium*, *Alternaria*, *Saprophyte spp.*) with a low infection rate of 0.01-0.19%. Chemical treatment with concentrations of 1%, 2% and 3% had a significant effect on the *Fusarium spp.* infection level, which was higher than the control, which was 0.18%, 0.17% and 0.19%.

Key words: Oil seed crop, Soybean production, Diseases, Ingredient difenoconazole, fungicide, physical therapy, soaking fungicides

Introduction

Soybean (*Glycine max* L.) is an annual oil seed crop that belongs to the order Fabales, family Fabaceae and is mostly known as edible oil. Soybean plants can grow up to 1.5 meters in length which are depending on their varieties (Singh *et al*., 2006). Soybean is a prominent nitrogen-fixing leguminous crop cultivated for both sustenance and livestock fodder, yields essential products such as soymilk, soymeal and soybean oil, which constitute significant components of human consumption(Alemu *et al*., 2014). The two key seed components that make soybean a significant crop are protein and oil. It has between 40 and 42 percent high-quality protein and between 18 and 22 percent oil with 85 percent unsaturated fatty acids. In addition to having high-quality protein, soybeans have a substantially larger protein concentration than other plant-based diets. This priceless bean has a carbohydrate content of roughly 12%. Soy protein is regarded as a

healthy protein since it provides a balanced amount of each of the important and required amino acids that the human body requires (Potter *et al.*, 1998). More than 2% of the world's soybeans are produced by the top five nations, namely the United States, Brazil, Argentina, China and India (FAO). In 2016 soybean crop was planted in an area of 120.48 million hectares and production is 351.74 million metric tons in the world. In 2017-18 world-wide production of soybean was about 347.4 million metric tons(Khurshid *et al.*, 2017). High-quality, certified seeds are crucial for optimizing soybean yield, with pathologically pristine seeds being a pivotal aspect of seed quality. Seed-borne pathogens, encompassing fungi, bacteria, viruses and nematodes, pose a threat, potentially inhabiting seed tissues or surfaces. Fungal inoculum may originate from various sources such as fields, seed handling processes, or distribution, compromising seed weight and viability (Ramdan *et al.*, 2021). Seed-borne fungi are a common occurrence in agricultural settings and can affect various crops. They are fungal pathogens that reside within or on the surface of seeds and can be transmitted from one generation of plants to the next. The occurrence of seed-borne fungi can vary depending on several factors, including the type of crop, environmental conditions, farming practices and seed quality (Martin *et al.*, 2022). Seed fungus infestation poses risks during growth, storage and germination, leading to reduced viability and seedling infection. Harvesting methods like seed falling on nets or ground increase susceptibility to seed-borne fungi, while mishandling during extraction creates conducive environments for fungal growth and seed damage. Improper temperature and moisture control during storage may facilitate fungal proliferation, while cool, damp nursery conditions further promote fungal growth and associated damage (Mehrotra *et al.*, 2003). Seed damage or seedling infection due to reduced viability results from fungal contamination, exacerbated by inadequate handling during processing and harvesting, facilitating fungal growth. Inappropriate temperature and moisture levels during storage contribute to fungal proliferation, particularly in cool, damp conditions during nursery seeding, ultimately causing harm (Amza *et al* ., 2018). The aim of following studies was to detection of Seed Borne Fungi Associated with Glycine max and its Management Through Physio–Chemical Treatments.

Material and Methods

Collection of Infected Seed Samples

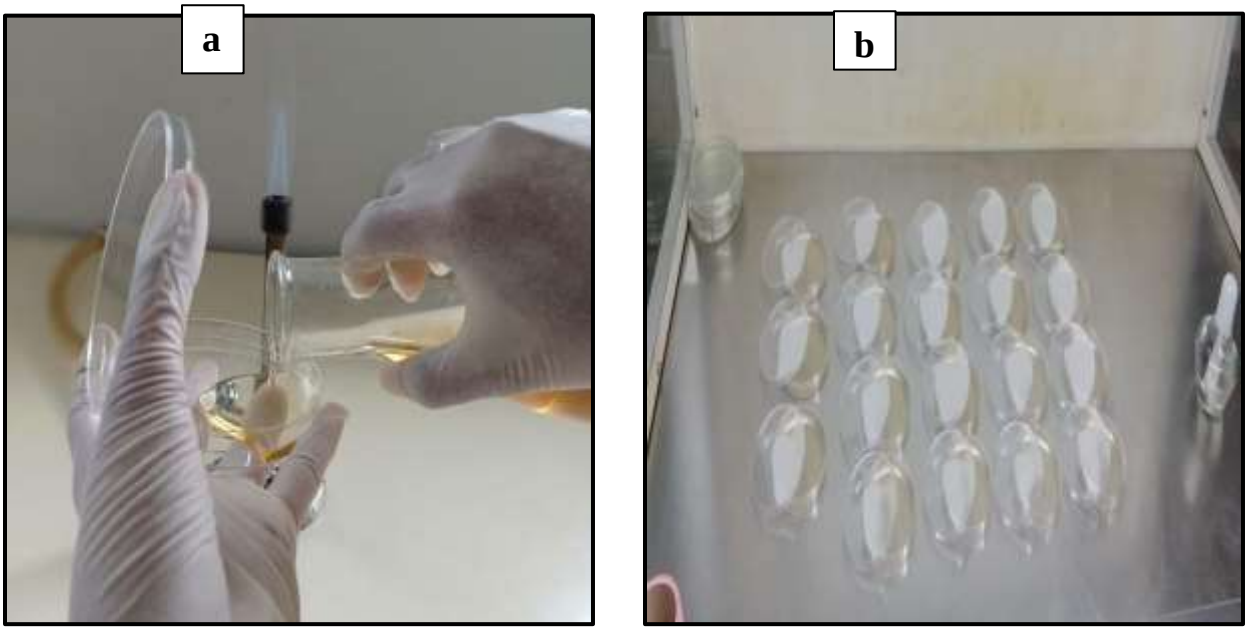
Five infected soybean varieties seed samples (Swat 84, Faisal, Ajmeri, Malakand, SL- 80) were collected showing the symptoms of discoloration and shriveling were selected. The isolation of pathogens from these infected seed samples was carried out in Plant Pathology Lab, UAF, Faisalabad. Diseased seeds were then collected in polythene bags and labeled by sample name.

Isolation of Pathogen from Infected Seed Samples

The agar plate method was used for the isolation process (PDA). After sterilizing soybean seeds for 1 minute with 70% ethanol, followed by 10% sodium hypochlorite for 1 minute, the seeds were then rinsed with sterile distilled water. The seeds were then placed onto sterile petri plates containing Potato Dextrose Agar, 3 seeds per plate and then plates were incubated at 24°C for a week (Ahmet *et al.*, 2021).

Potato Dextrose Agar (PDA) Media Preparation

A one-liter batch of Potato Dextrose Agar (PDA) was prepared using the following constituents: 220g of potato starch, 20g of agar-agar, 20g of dextrose, and 1000ml of distilled water. Fresh potatoes weighing 200g were peeled, diced, and boiled in 500ml of distilled water. The resultant solution was filtered through muslin cloth and transferred to a 2-liter container. Subsequently, 20g of glucose and 20g of agar were added, followed by gentle agitation to ensure homogeneity. Additional distilled water was incorporated to achieve a final volume of 1 liter, and the container was sealed. Sterilization was accomplished through autoclaving at 121°C for 20 minutes at 15 psi to eradicate contaminants. The sterilized medium was aseptically dispensed into 9 cm petri plates for further utilization (PLATE and ZAKI, 2018).



Freshly prepared PDA Media plates

Purification and Identification of Fungi

Isolated fungal pathogens were cultured on separate PDA plates and examined under a compound microscope. Identification involved comparing their morphological features like mycelium appearance and spore characteristics with published data (Kunjam *et al.*, 2019).

Germination Test

Healthy soybean seeds were artificially inoculated by sterilizing their surface with a 70% ethanol solution followed by immersion in a spore suspension for 15-20 minutes. The inoculated seeds were placed on moist filter paper in Petri plates and incubated. Fungal growth and seed germination were observed using a compound microscope. The contamination rate (CR) in soybean seeds was calculated according to the Equation:

$$\mathbf{CR = NIS/TNS}$$

Where NIS is the number of infected seeds and TNS is the total number of seeds (Ustun *et al.*, 2021). The Percentage of Germination (PG) for soybean seed varieties was calculated as the ratio of germinated seeds to the total seed count, employing the specified formula.

$$\mathbf{PG = NSG/\ NTS \times 100}$$

Where NSG is the number of seeds germinated and NTS is the number of total seeds (Kunjam *et al.*, 2019).

Chemical Treatment

Chemical treatment was employed in two ways. First, the isolated fungal pathogen was grown on media poisoned with a suitable fungicide (Poisoned food technique).

Poisoned food technique

The antifungal potential of Dumortiera extract was assessed against Fusarium spp. using the poisoned food technique (Grover and Moore, 1962). Autoclaved PDA was utilized as the medium, with varying concentrations of diphenacanazole (100ppm, 200ppm, and 300ppm) prepared by dilution in distilled water. Control sets received distilled water instead of extract. Fungal growth was observed on the 7th day of incubation at 25±2^{o}C.

Healthy soybean seeds underwent artificial inoculation by surface sterilization using a 70% ethanol solution, followed by immersion in a spore suspension for 15-20 minutes. These inoculated seeds were then placed on moist filter paper in Petri plates and incubated. Fungal growth and seed germination were monitored using a compound microscope. The contamination rate (CR) in soybean seeds was determined using the following equation. (Ramdan *et al.*, 2021).

Physical Treatment:

Five soybean varieties (Swat 84, Faisal, Ajmeri, SL-80, Malakand) were subjected to physical treatments involving exposure to hot air at 40°C for durations of 10, 20, and 30 seconds, alongside chemical treatments. Seed germination evaluations were performed in pots containing sterilized soil, with data collection at 7, 14, and 21 days post-treatment.

Soybean seed germination in sterilized pots

Observation

Data were recorded on the following parameters.

Plant height (cm)

The plant height (PH) was measured by spreading the whole plant in a straight position on the clipboard. The meter rod measured the height in centimeters and the average was calculated to get accuracy in the recording data

Root length (cm)

Root length (RL) was measured from the point of cotyledons to the end of the roots. A meter rod was used to measure the length in centimeters and the average was calculated.

Shoot length (cm)

Shoot length (SL) was measured by using a meter rod in centimeters. It was measured above the point of cotyledons to the upper most tip by spreading the plant in a fully straight position and the average was worked out.

Root shoot ratio

Root shoot ratio (R/S) was calculated by dividing the root length by the shoot length of the selected plants.

Fresh root weight (g)

The plants were used to record the fresh root weight (FRW) quickly after harvesting Scissors were used to isolate the root from the shoot at the junction of cotyledons and the average was worked out to get precision.

Germination %

Germination percentage can be calculated by the following formula:

Germination percentage =No of seeds germinated /total no of seeds × 100

Fresh shoot weight /(g)

Fresh shoot weight (FSW) was measured using digital weight balance. The average was calculated by repeating the readings, this process was performed quickly, to save the original water content in the plants

Dry root weight (g)

Dry root weight (DRW) was recorded after oven-drying at 65. C for 48 h. A digital weight balance was used to record the weight and the average was worked out.

Dry shoot weight (g)

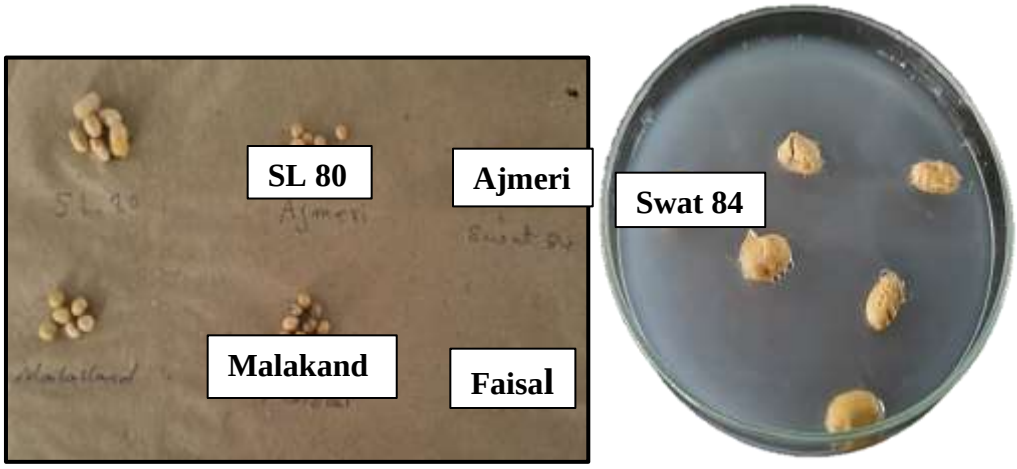
Dry shoot weight (DSW) was measured by using the same methods as mentioned above in the case of DRW.

Disease incidence

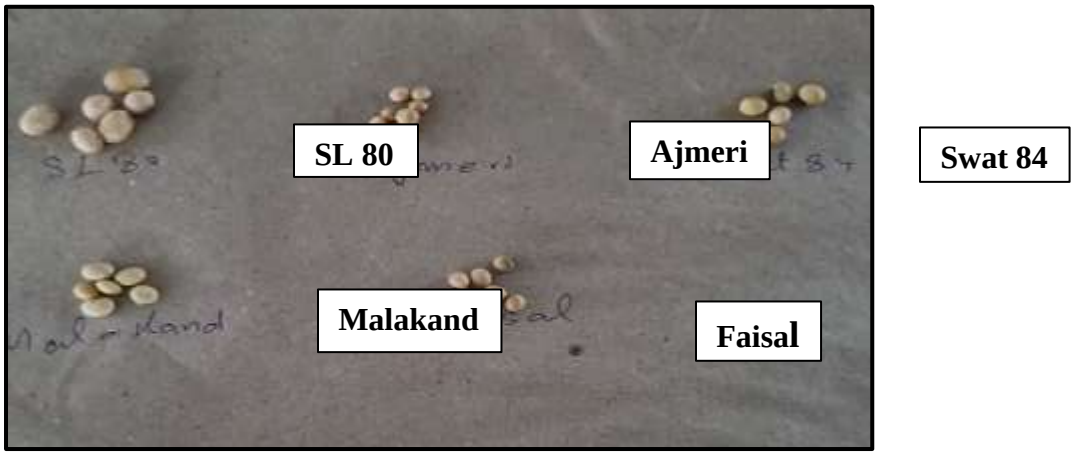
The disease incidence of soya bean seeds was determined as a proportion of infected plants over the total number of plants by the following formula:

Disease incidence %= No. of diseased plants /Total No. of plants x 100

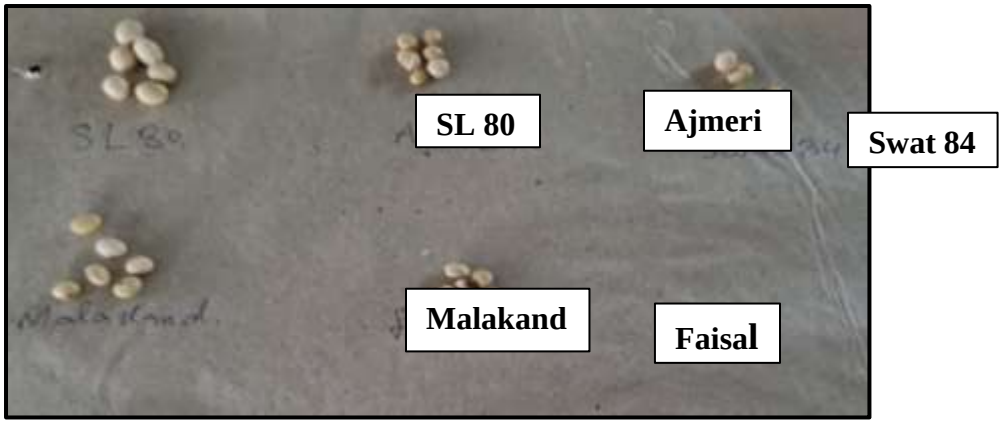
(Inam *et al.*,2012)



Chemical treatment of seeds via priming in fungicide (difenoconazole) with 1% concentration



Chemical treatment of seeds via priming in fungicide (difenoconazole) with 2% concentration



Chemical treatment of seeds via priming in fungicide (difenoconazole) with 3% concentration

Statistical Analysis of Data

Each treatment had three replications and the whole experiment was planned to use a complete randomized design (CRD). The data were recorded four times within seven days of intervals. The data were analyzed by ANOVA estimation using Statistics (Steels *et al*., 1997).

RESULTS AND DISCUSSION

Fungi species isolated soybean seed varieties

A total of 41 isolates identified as three genera and seven species were obtained from 5 soybean seed varieties. The most common genera across three growing regions were *Fusarium* (65.8%), *Alternaria* (21.9%) and *Penicillium* (12.1%) were identified. *Fusarium spp.* (61.77%) was the most common species followed by *Alternaria spp.* (29.1%) and *Saprophyte spp*. with a frequency of 10.3%. (Fig 4.1).

Fungi isolated from soybean seeds on PDA plates

4.1.1 *Fusarium spp.*

Fusarium species were found in significant frequencies on the soybean seed varieties of Malakand (38.46%) and Faisal (26.32%). A percentage of 7.7 from SL80 and 15.8 from Ajmeri of the total isolations were identified at the genus level as *Fusarium spp*. On the PDA agar plate, *Fusarium* colonies had floccose mycelium with a white to pale salmon color that became brown with age as shown in (Fig.4.2)

Fungi (*Fusarium spp.*) isolated from soybean seeds on PDA plates

Alternaria spp.:

Alternaria spp. was isolated from SL80, Ajmeri, Malakand and Swat-84 with a frequency of 7.7%, 24.8%, 10% and 15.8%, respectively. In this study, colonies of *Alternaria spp*. had a yellow-brown mycelium covering the whole plate (Fig.4.3).

Fungi (*Alternaria spp*.) isolated from soybean seeds on PDA plates

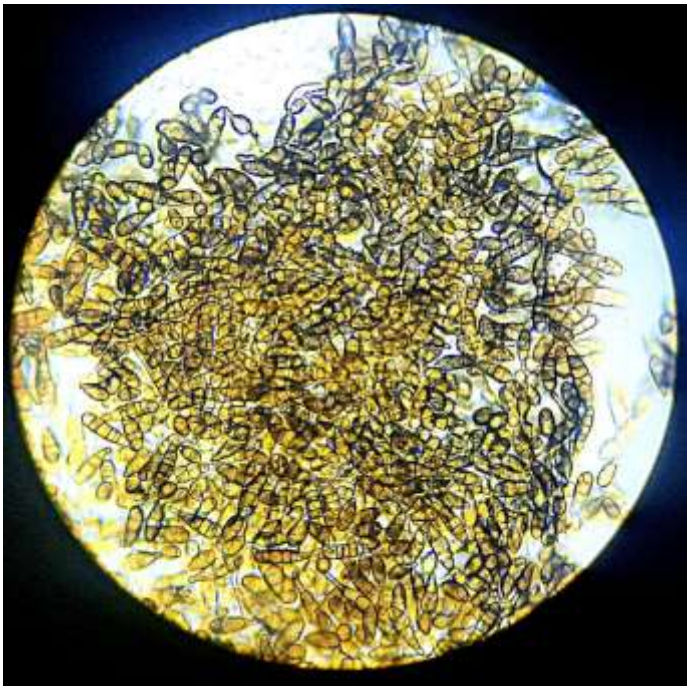
Saprophyte:

Saprophyt**e** was isolated from SL80, Ajmeri, Malakand and Swat-84 with a frequency of 5.7%, 20.8%, 10% and 11.8%, respectively. In this study, colonies of Saprophyte are *g*reen or green-gray. It had a greenish mycelium covering the whole plate

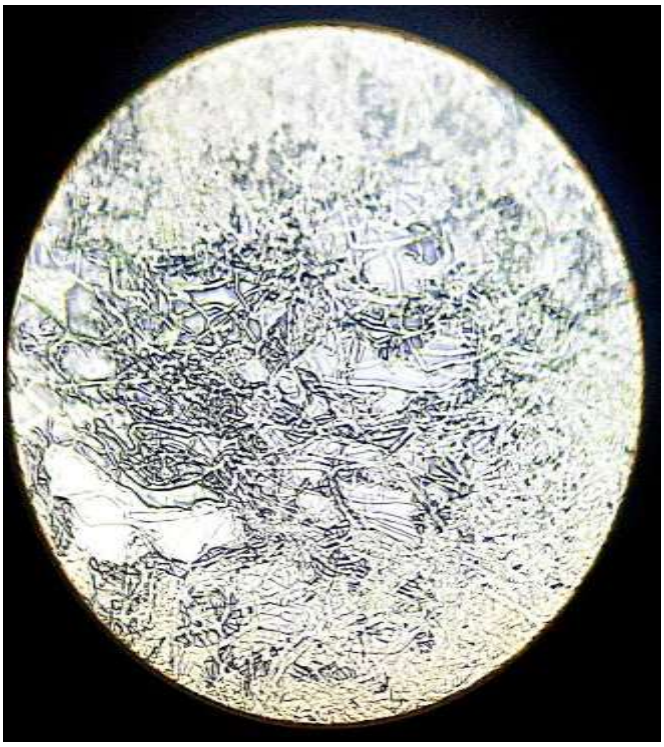
Fungi (*Saprophyte spp.*) isolated from soybean seeds on PDA plates

Seed-borne Pathogen Identification:

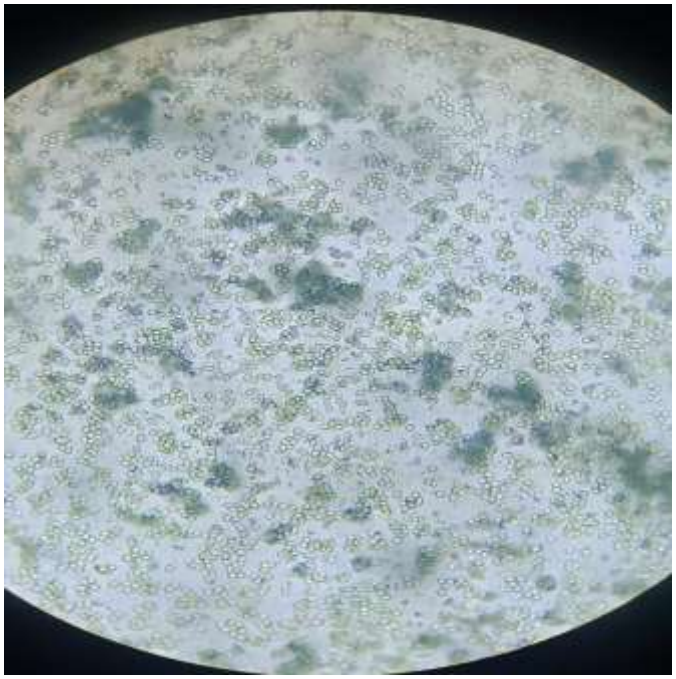
The pathogens carried by soybean seeds that were identified consisted of 3 groups of fungi (*Fusarium spp*, *Alternaria spp*, *Saprophyte spp.)*. Identification was carried out by observing the morphology microscopically. The identification results were microscopic morphological features and fungal colonies on the surface of the seeds.



Microscopic identification of Fungi associated with soya bean seeds (*Alternaria spp*)



Microscopic identification of Fungi associated with soya bean seeds (*Fusarium spp)*



Microscopic identification of Fungi associated with soya bean seeds (Saprophyte *spp.*)

4.3 Percentage of Germination (PG) of soybean seeds varieties:

A germination test was performed on healthy soybean seed varieties. From a total of 50 healthy soybean seeds, 47 seeds germinated. Out of 47 germinated seeds, 41 were found infected**.** 27 were infected with *Fusarium spp.* and **9** were infected by *Alternaria spp.* and 5 were infected by other fungal species. The percentage of Germination (PG) of soybean seeds varieties was determined as a proportion of germinated seeds over the total number of seeds and computed by using the following formula:

Where NSG is the number of seeds germinated and NTS is the number of total seeds.

Total Number of seeds = 50

No. of germinated seeds = 47

Percentage of Germination (PG = NSG/ NTS × 100

PG= 47/50× 100

Percentage of Germination = **94.0%**

4.4 Contamination rate (CR of soybean seeds varieties:

The contamination rate (CR) in soybean seeds was calculated according to the Equation:

$$\mathbf{CR = NIS/TNS \times 100}$$

Where NIS is the number of infected seeds and TNS is the total number of seeds.

Total number of germinated seeds (TNS)= 47

No of infected seeds (NIS)= 41

$$\mathbf{CR = 41/47 \times 100}$$

$$\mathbf{Contamination\ Rate\ (CR) = 87.23\%}$$

No of *Fusarium spp.* infected seeds =27

No of *Alternaria spp.* infected seeds =9

CR OF *FUSARIUM SPP.* = 27 ÷41×100 = **65.85 %**

CR OF *ALTERNARIA SPP.* = 9 ÷41×100 = **21.90 %**

Table 4.1 Seed Germination and Contamination Rate of infected Soybean varieties:

Fungi Species	SG (Seed Germination)	CR (Contamination Rate)
Fusarium spp.	94.0%	65.85 %
Alternaria spp.	94.0 %	21.90 %

Fig 4.6: Seed germination on plates contaminated with fungi

4.5 Chemical Treatment

4.5.1 Food poisoning technique

The poisoned food technique is utilized for evaluating the antifungal potential of plant extracts. In this method, agar in a petri dish is inoculated with a test fungus and subsequently overlaid with a solution containing the plant extract. The dish is then incubated at an optimal temperature for the test fungus' growth. Percentage inhibition of mycelial growth represents the proportion of the test fungus hindered from growing by the plant extract, while the Minimum Inhibitory Concentration (MIC) denotes the lowest extract concentration inhibiting all test fungi growth. Data analysis reveals varying antifungal efficacy among tested plant extracts. Notably, the extract from *Decalepis hamiltonii* exhibits the most potent antifungal activity, inhibiting growth of all test fungi at a concentration of 10%. Moreover, the concentration of the plant extract significantly influences its antifungal effectiveness, with higher concentrations correlating with increased inhibition of mycelial growth.

Analysis of Variance of Food Poisoned Technique

Source	DF	SS	MS	F	P
Treatment	1	4360.39	4360.39	129.21**	0
Concentra	2	1409.71	704.86	20.89**	0
Days	2	1698.48	849.24	25.17**	0
Treatment*Concentra	2	947	473.5	14.03**	0
Treatment*Days	2	1021.02	510.51	15.13**	0
Concentra*Days	4	488.69	122.17	3.62*	0.011
Treatment*Concentra*Days	4	458.48	114.62	3.4*	0.015
Error	54	1822.33	33.75		
Total	71				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Growth for Concentration

Concentra	Mean	Homogeneous Groups
3	42.344	A
2	39.072	A
1	32.059	B

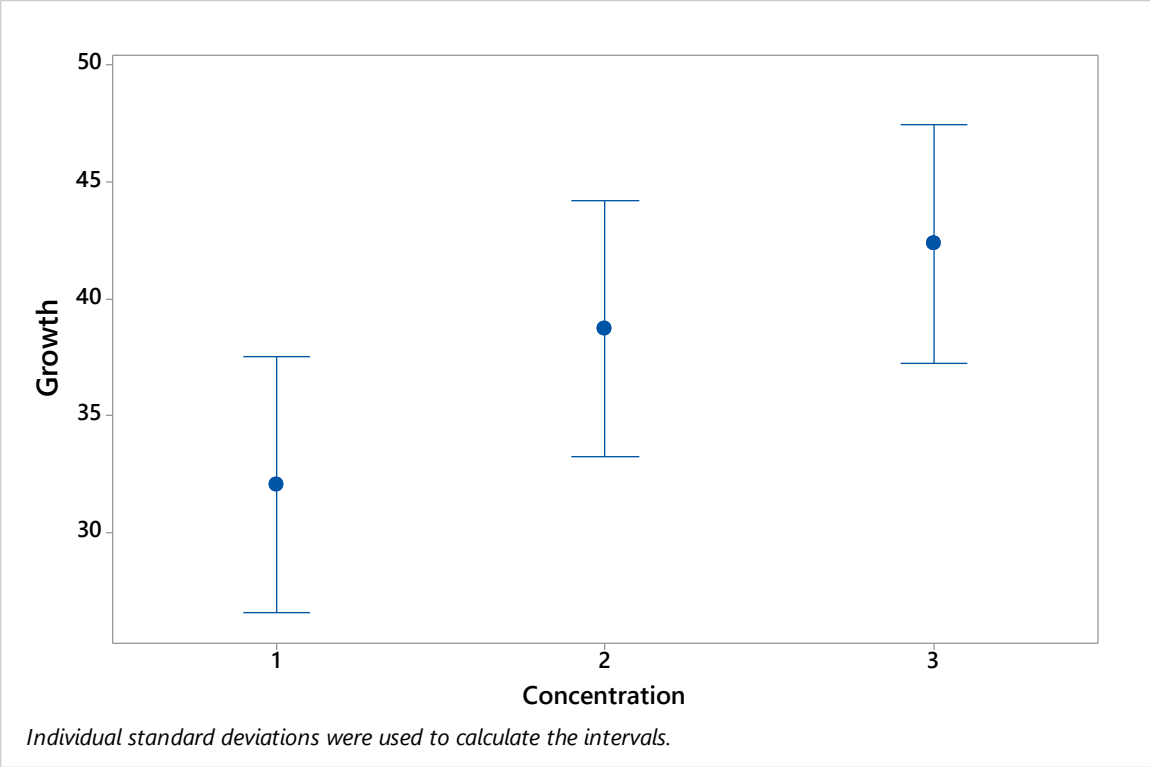


Fig 4.7: Effect of the concentration of the Food Poisoning Technique

LSD All-Pairwise Comparisons Test of Growth for Day

Days	Mean	Homogeneous Groups
3	44.026	A
2	37.646	B
1	31.803	C

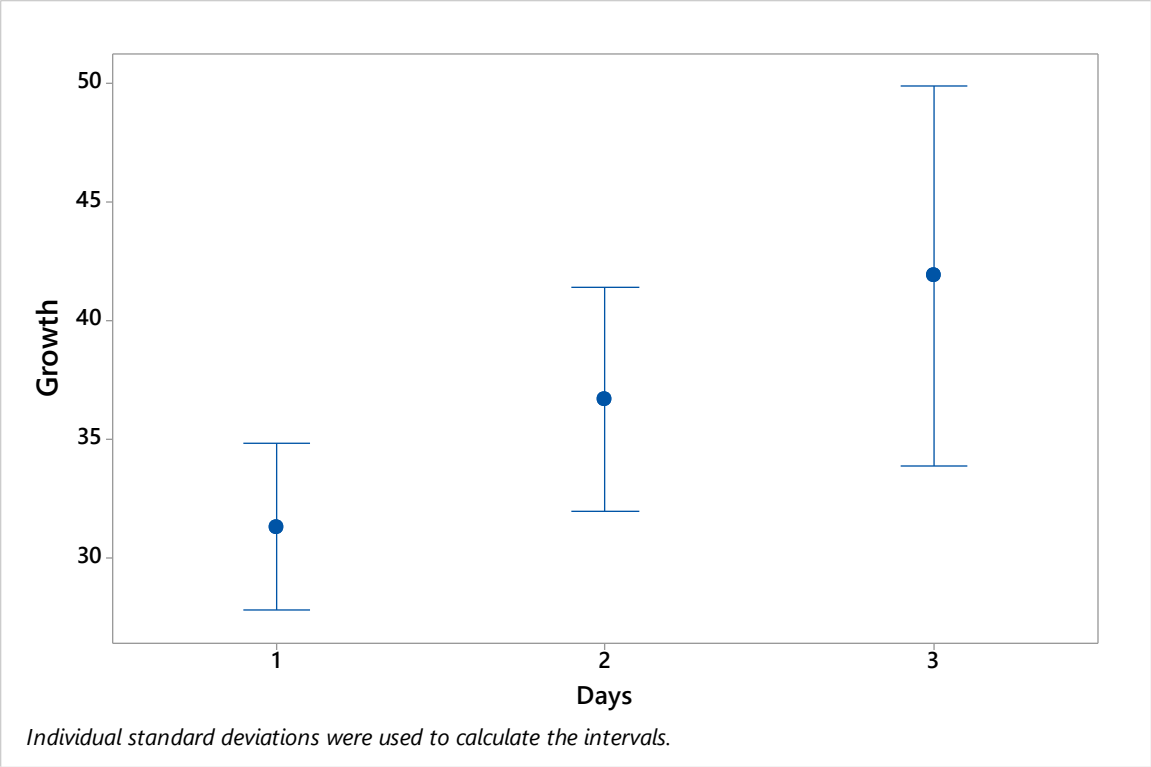


Fig 4.8: Effect of the Days of the Food Poisoning Technique

4.5.2 Results of Chemical Treatment

4.5.2.1 Plant height

The plant height of five different varieties of soybean (V1-V5) under three different concentrations of difenoconazole (C0-C3). The results show that plant height increases with increasing concentration of difenoconazole. For example, the average plant height of variety V1 (Swat 84) is 25.32 cm under control conditions (C0), but it increases to 27.36 cm under 1% concentration (C1), 31.23 cm under 2% concentration (C2) and 34.36 cm under 3% concentration (C3). The results also show that there is a significant difference in plant height between varieties. For example, the average plant height of variety V1 (Swat 84) is 25.32 cm under control conditions, but the average plant height of variety V5 (Malakand) is only 20.35 cm under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to difenoconazole than variety V5 (Malakand).

Analysis of Variance Table for Plant Height

Source	DF	SS	MS	F	P
Treatment	3	413.889	137.963	135.58**	0
GENO	4	276.997	69.249	68.05**	0
treatment*GENO	12	14.179	1.182	1.16ns	0.3527
Error	30	30.527	1.018		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Plant Height for Treatment

Treatment	Mean	Homogeneous Groups
3	30.067	A
2	26.647	B
1	23.327	C
0	22.648	C

LSD All-Pairwise Comparisons Test of Plant Height for Geno

GENO	Mean	Homogeneous Groups
1	29.572	A
2	27.262	B
3	25.726	C
4	23.771	D
5	22.029	E

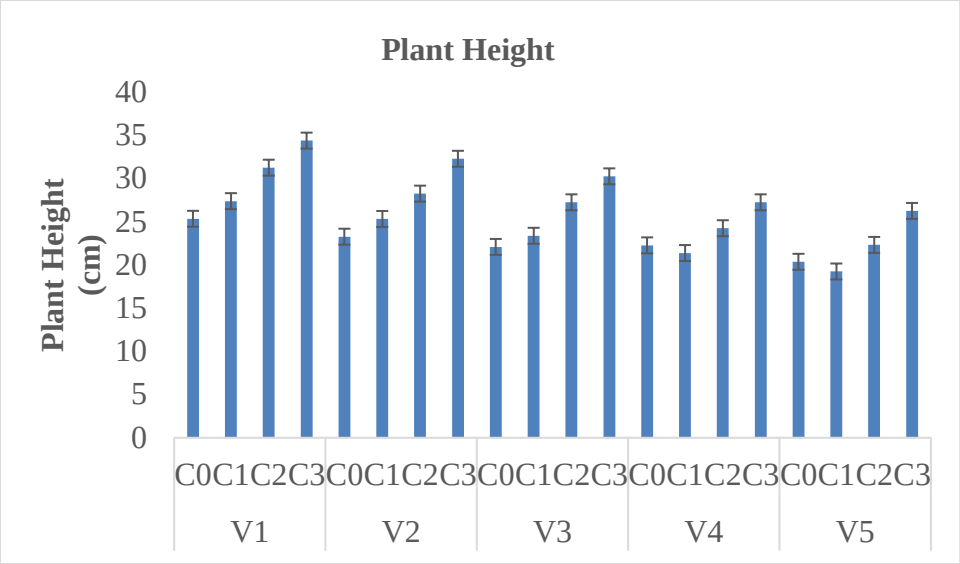


Fig.4.9: Graph showing Plant Height (cm) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4=SL-80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.2 Root length

The root length of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that root length increases with increasing concentration of the fungicide. For example, the average root length of variety V1 (Swat 84) is 10.25 cm under control conditions (C0), but it increases to 11.3 cm under 1% concentration (C1), 12.33 cm under 2% concentration (C2) and 13.3 cm under 3% concentration (C3). The results also show that there is a significant difference in root length between varieties. For example, the average root length of variety V1 (Swat 84) is 10.25 cm under control conditions, but the average root length of variety V5 (Malakand) is only 8.35 cm under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root Length

Source	DF	SS	MS	F	P
Treatment	3	82.4001	27.4667	98.49**	0
GENO	4	43.0538	10.7634	38.59**	0
Treatment*GENO	12	3.2885	0.274	0.98ns	0.4865
Error	30	8.3667	0.2789		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root Length for Treatment

Treatment	Mean	Homogeneous Groups
3	26.867	A
2	25.307	B
1	24.207	C
0	22.93	D

LSD All-Pairwise Comparisons Test of Root Length for GENO

GENO	Mean	Homogeneous Groups
1	25.979	A
2	25.45	B
3	25.258	B
4	24.45	C
5	23	D

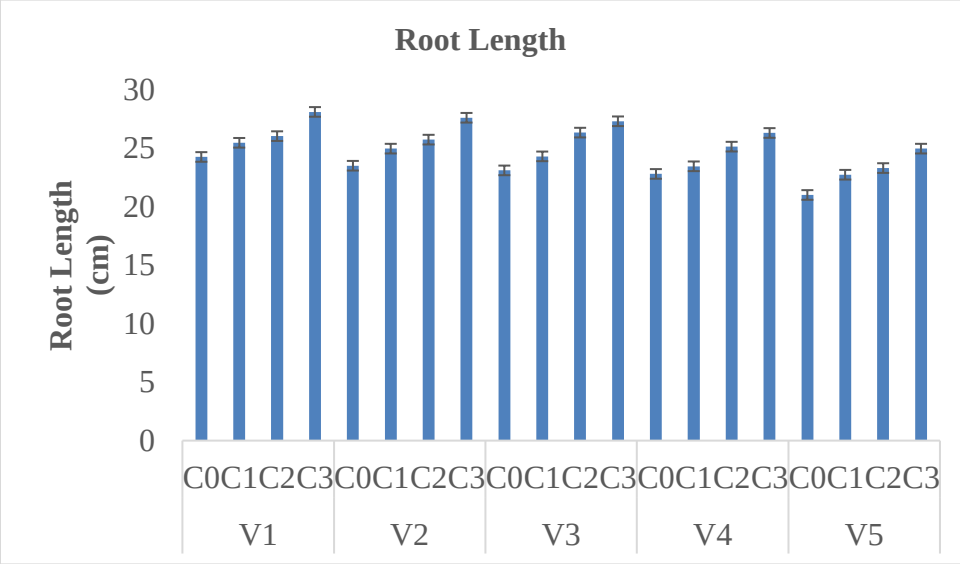


Fig: 4.10: Graph showing Root Length (cm) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.3 Shoot Length

The shoot length (SL) and root length (RL) of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that both shoot length and root length increase with increasing concentration of the fungicide. For example, the average shoot length of variety V1 (Swat 84) is 24.25 cm under control conditions (C0), but it increases to 25.3 cm under 1% concentration (C1), 26.33 cm under 2% concentration (C2) and 27.3 cm under 3% concentration (C3). The average root length of variety V1 (Swat 84) is 10.25 cm under control conditions (C0), but it increases to 11.3 cm under 1% concentration (C1), 12.33 cm under 2% concentration (C2) and 13.3 cm under 3% concentration (C3). The results also show that there is a significant difference in both shoot length and root length between varieties. For example, the average shoot length of variety V1 (Swat 84) is 24.25 cm under control conditions, but the average shoot length of variety V5 (Malakand) is only 20.35 cm under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Shoot Length

Source	DF	SS	MS	F	P
Treatment	3	515.141	171.714	2006.42**	0
GENO	4	78.79	19.697	230.16**	0
treatment*GENO	12	1.107	0.092	1.08ns	0.4113
Error	30	2.567	0.086		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Shoot length for treatment

Treatment	Mean	Homogeneous Groups
3	32.319	A
2	26.45	B
1	25.444	C
0	23.32	D

LSD All-Pairwise Comparisons Test of Shoot Length for GENO

GENO	Mean	Homogeneous Groups
1	29.057	A
2	27.6	B
3	26.875	C
4	25.917	D
5	24.967	E

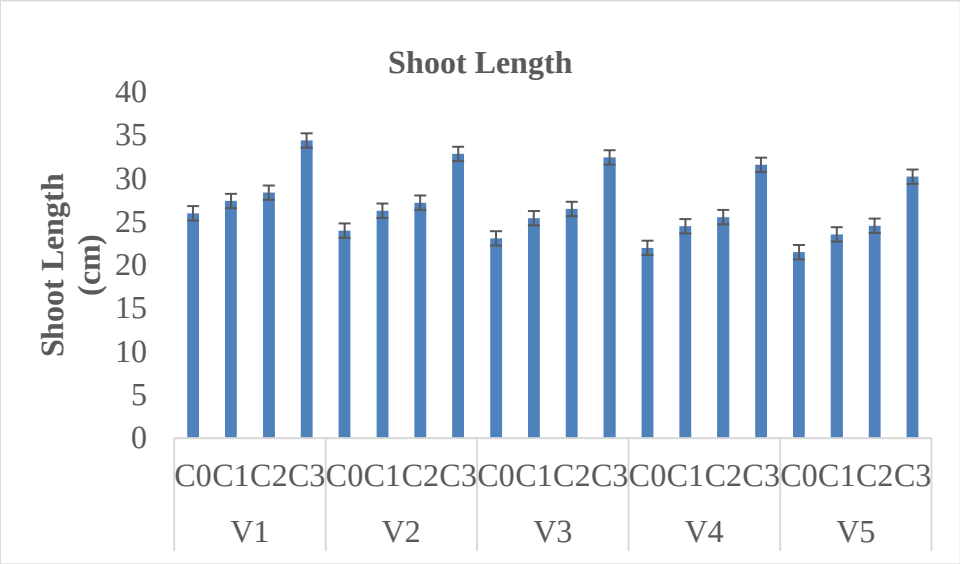


Fig 4.11: Graph showing Shoot Length (cm) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.4 Root Fresh Weight:

The root fresh weight (RFW) of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that RFW increases with increasing concentration of the fungicide. For example, the average RFW of variety V1 (Swat 84) is 23.1 g under control conditions (C0), but it increases to 27.16 g under 1% concentration (C1), 29.76 g under 2% concentration (C2) and 34.68 g under 3% concentration (C3). The results also show that there is a significant difference in RFW between varieties. For example, the average RFW of variety V1 (Swat 84) is 23.1 g under control conditions, but the average RFW of variety V5 (Malakand) is only 20.35 g under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root Fresh Weight

Source	DF	SS	MS	F	P
Treatment	3	725.983	241.994	15782.2**	0
GENO	4	45.448	11.362	740.99**	0
treatment*GENO	12	2.16	0.18	11.74**	0
Error	30	0.46	0.015		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root Fresh Weight for Treatment

treatment	Mean	Homogeneous Groups
3	33.409	A
2	28.319	B
1	25.351	C
0	21.912	D

LSD All-Pairwise Comparisons Test of Root Fresh Weight for GENO

GENO	Mean	Homogeneous Groups
1	28.678	A
2	27.961	B
3	27.447	C
4	26.492	D
5	25.661	E

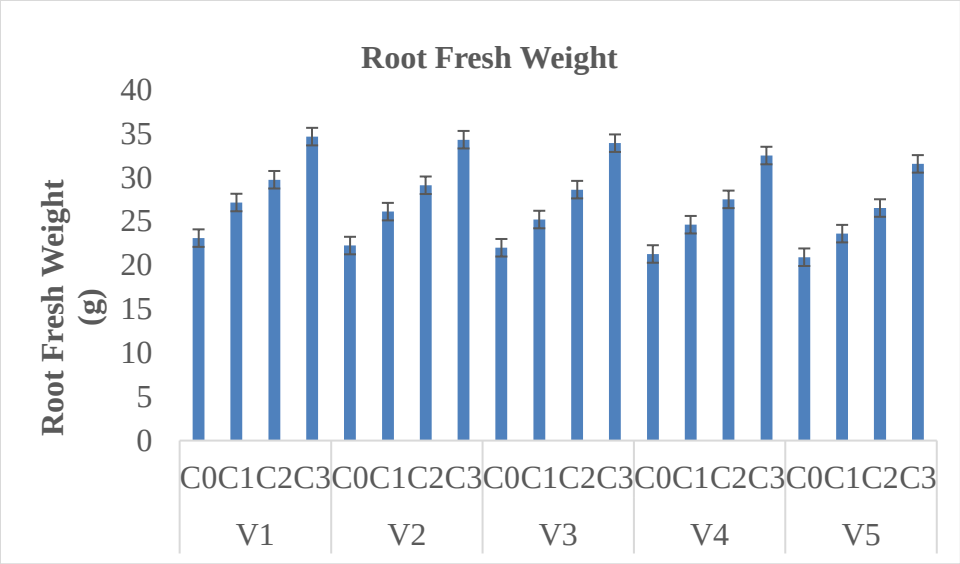


Fig.4.12: Graph showing Root Fresh Weight (g) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.5 Root Dry Weight:

The root dry weight (RDW) of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that RDW increases with increasing concentration of the fungicide. For example, the average RDW of variety V1 (Swat 84) is 4 g under control conditions (C0), but it increases to 5.5 g under 1% concentration (C1), 7.15 g under 2% concentration (C2) and 8.14 g under 3% concentration (C3). The results also show that there is a significant difference in RDW between varieties. For example, the average RDW of variety V1 (Swat 84) is 4 g under control conditions, but the average RDW of variety V5 (Malakand) is only 2.85 g under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root Dry Weight

Source	DF	SS	MS	F	P
Treatment	3	78.9475	26.3158	23357.3**	0
GENO	4	9.5296	2.3824	2114.55**	0
treatment*GENO	12	1.8385	0.1532	135.98**	0
Error	30	0.0338	0.0011		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root Dry Weight for Treatment

Treatment	Mean	Homogeneous Groups
3	7.3233	A
2	6.3387	B
1	5.02	C
0	3.288	D

LSD All-Pairwise Comparisons Test of Root Dry Weight for GENO

GENO	Mean	Homogeneous Groups
1	6.2133	A
2	5.9433	B
3	5.1792	C
4	5.0933	D
5	5.0333	E

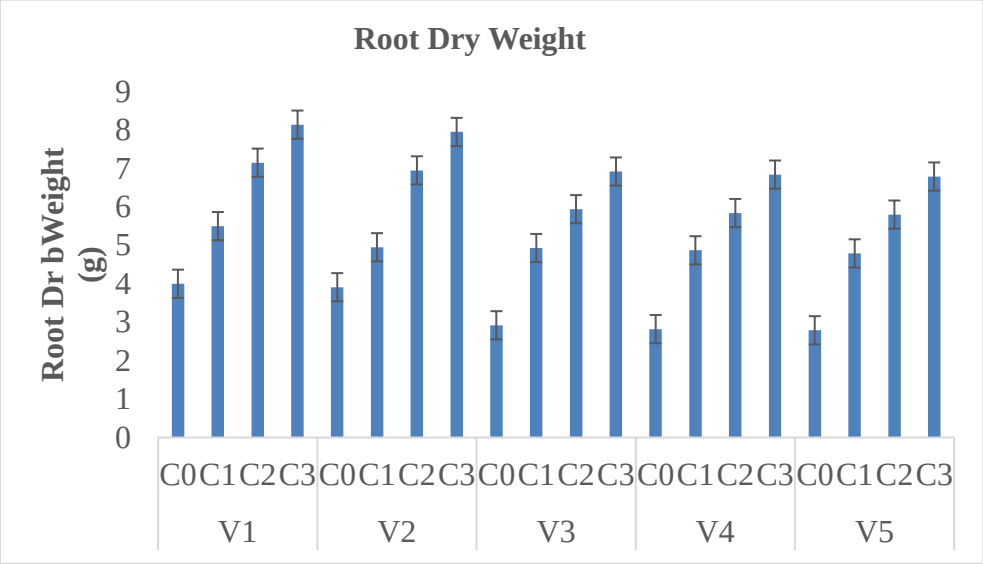


Fig.4.13: Graph showing Root Dry Weight (g) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.6 Shoot Fresh Weight:

The shoot fresh weight (SFW) of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that SFW increases with increasing concentration of the fungicide. For example, the average SFW of variety V1 (Swat 84) is 16 g under control conditions (C0), but it increases to 18.5 g under 1% concentration (C1), 21.25 g under 2% concentration (C2) and 24.14 g under 3% concentration (C3). The results also show that there is a significant difference in SFW between varieties. For example, the average SFW of variety V1 (Swat 84) is 16 g under control conditions, but the average SFW of variety V5 (Malakand) is only 13.85 g under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Shoot Fresh Weight

Source	DF	SS	MS	F	P
Treatment	3	229.294	76.4312	22960**	0
GENO	4	8.01	2.0025	601.54**	0
treatment*GENO	12	3.653	0.3044	91.45**	0
Error	30	0.1	0.0033		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Shoot Fresh Weight for Treatment

Treatment	Mean	Homogeneous Groups
3	31.917	A
2	28.888	B
1	27.507	C
0	25.31	D

LSD All-Pairwise Comparisons Test of Shoot Fresh Weight for GENO

GENO	Mean	Homogeneous Groups
1	28.937	A
2	28.706	B
3	28.606	C
4	28.056	D

5	27.723	E

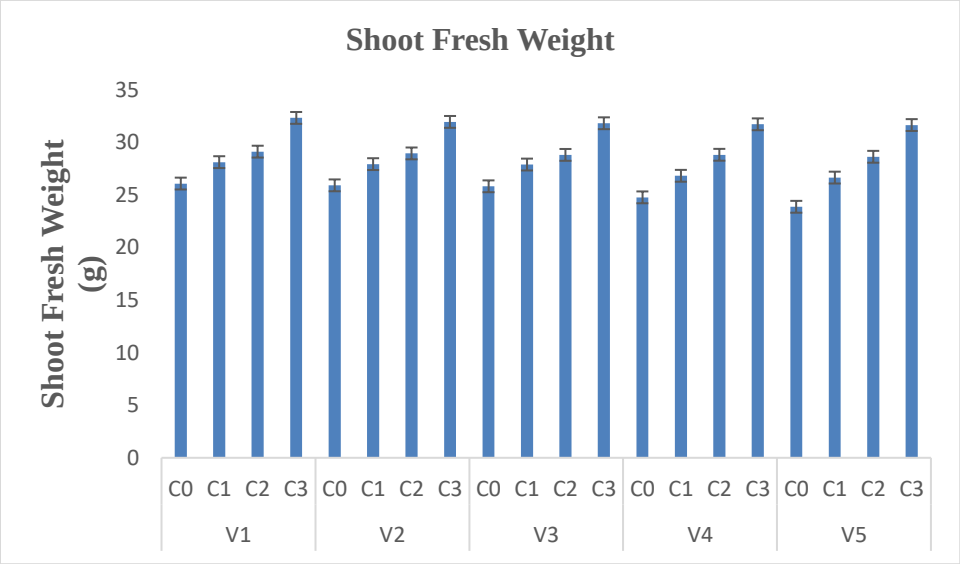


Fig.4.14: Graph showing Shoot Fresh Weight (g) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.7 Shoot Dry Weight:

The SDW of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that SDW increases with increasing concentration of the fungicide. For example, the average SDW of variety V1 (Swat 84) is 5 g under control conditions (C0), but it increases to 6.5 g under 1% concentration (C1), 8.15 g under 2% concentration (C2) and 9.14 g under 3% concentration (C3). The results also show that there is a significant difference in SDW between varieties. For example, the average SDW of variety V1 (Swat 84) is 5 g under control conditions, but the average SDW of variety V5 (Malakand) is only 3.85 g under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Shoot Dry Weight

Source	DF	SS	MS	F	P
Treatment	3	90.0821	30.0274	19058.3**	0
GENO	4	1.0154	0.2539	161.12**	0
treatment*GENO	12	0.0543	0.0045	2.87*	0.0094
Error	30	0.0473	0.0016		

Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Shoot Dry Weight for Treatment

Treatment	Mean	Homogeneous Groups
3	8.912	A
2	6.918	B
1	5.8973	C
0	5.158	

LSD All-Pairwise Comparisons Test of Shoot Dry Weight for GENO

GENO	Mean	Homogeneous Groups
1	6.9792	A
2	6.7992	B
3	6.7033	C
4	6.605	D
5	6.52	E

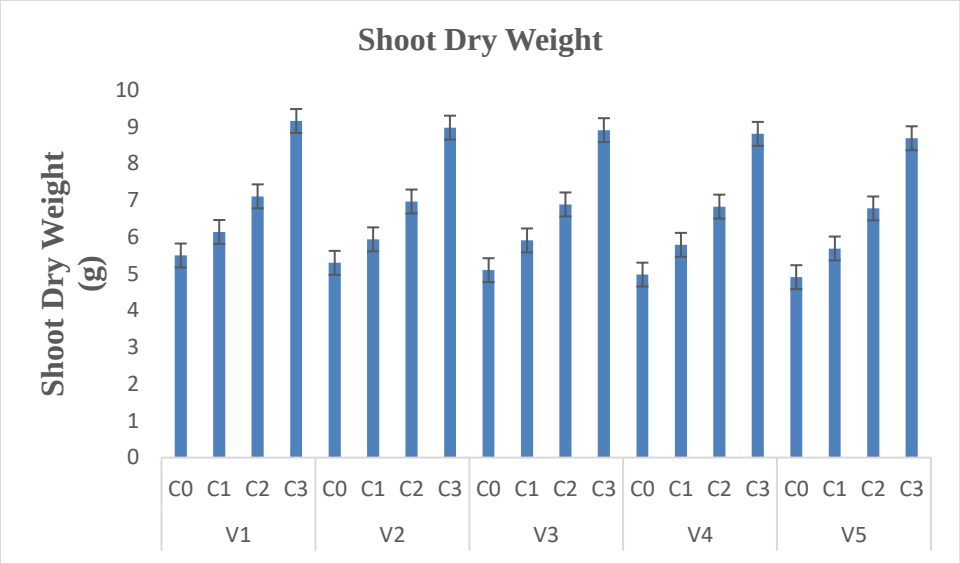


Fig.4.15: Graph showing Shoot Dry Weight (g) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.2.8 Root /Shoot Ratio:

Root shoot ratio of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that the R/S increases with increasing concentration of the fungicide. For example, the average R/S of variety V1 (Swat 84) is 0.5 under control conditions (C0), but it increases to 0.6 under 1% concentration (C1), 0.75 under 2% concentration (C2) and 0.84 under 3% concentration (C3). The results also show that there is a significant difference in R/S between varieties. For example, the average R/S of variety V1 (Swat 84) is 0.5 under control conditions, but the average R/S of variety V5 (Malakand) is only 0.38 under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root/ Shoot Ratio

Source	DF	SS	MS	F	P
Treatment	3	0.17535	0.05845	77.65**	0
GENO	4	0.01439	0.0036	4.78**	0.0042
treatment*GENO	12	0.00742	0.00062	0.82ns	0.6279
Error	30	0.02258	0.00075		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root/ Shoot for treatment

Treatment	Mean	Homogeneous Groups
0	0.985	A
2	0.9576	AB
1	0.9522	B
3	0.8315	C

LSD All-Pairwise Comparisons Test of Root/ Shoot Ratio for GENO

GENO	Mean	Homogeneous Groups
4	0.9536	A
3	0.9476	AB
5	0.9291	AB
2	0.9283	B
1	0.8993	C

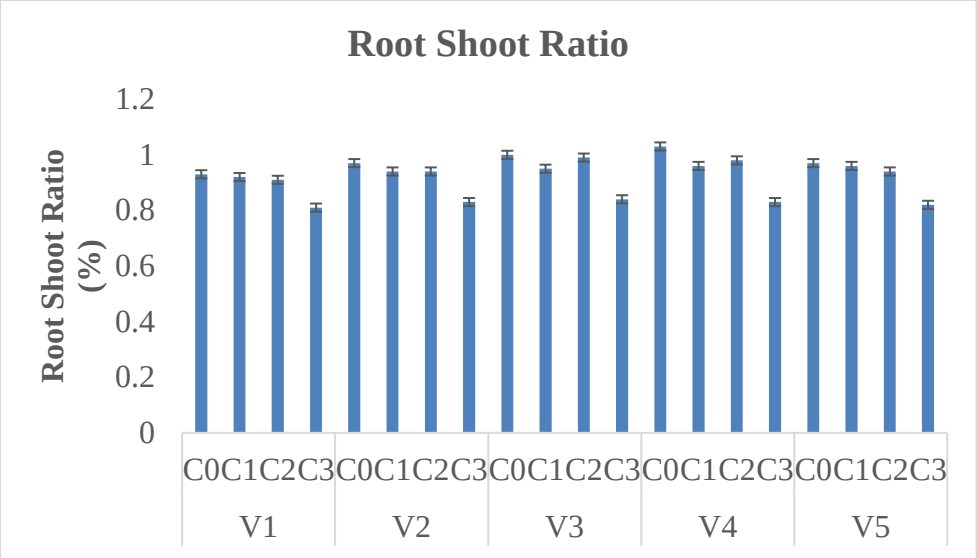


Fig. 4.16: Graph showing Plants Germination (%) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations

4.5.2.9 Germination Percentage

The germination percentage (GP) of five different varieties of soybean (V1-V5) under three different concentrations of fungicide (C0-C3). The results show that GP increases with increasing concentration of the fungicide. For example, the average GP of variety V1 (Swat 84) is 72% under control conditions (C0), but it increases to 80.23% under 1% concentration (C1), 89.19% under 2% concentration (C2) and 97.17% under 3% concentration (C3). The results also show that there is a significant difference in GP between varieties. For example, the average GP of variety V1 (Swat 84) is 72% under control conditions, but the average GP of variety V5 (Malakand) is only 63.23% under the same conditions. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Germination %

Source	DF	SS	MS	F	P
Treatment	3	3876.97	1292.32	30049.4**	0
GENO	4	123.74	30.93	719.29**	0
treatment*GENO	12	35.95	3	69.65**	0
Error	30	1.29	0.04		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Germination % for Treatment

Treatment	Mean	Homogeneous Groups
3	95.252	A
2	83.942	B
1	78.979	C
0	66.112	D

LSD All-Pairwise Comparisons Test of Germination % for GENO

GENO	Mean	Homogeneous Groups
1	83.64	A
2	82.59	B
3	80.415	C
4	79.707	D
5	79.005	

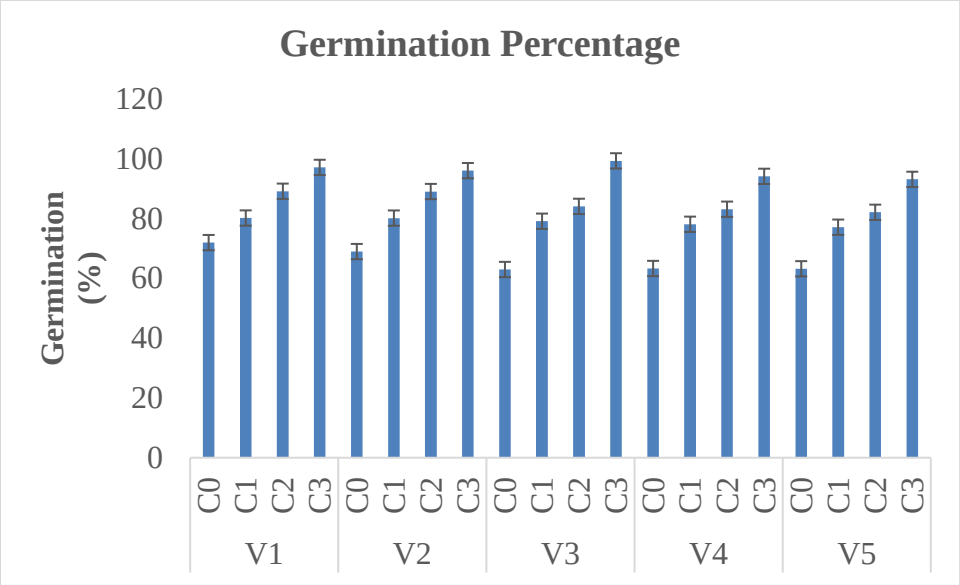


Fig.4.17: Graph showing Plants Germination (%) of Chemically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3 Results of Physical Treatment

4.5.3.1 Plant height

The plant height of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in plant height. For example, the average plant height of variety V1 (Swat 84) is 25.32 cm under control conditions (C0), but it increases to 27.36 cm under 1% concentration (C1), 31.23 cm under 2% concentration (C2) and 34.36 cm under 3% concentration (C3). On the other hand, the average plant height of variety V5 (Malakand) is only 20.35 cm under control conditions and it only increases to 22.39 cm, 25.35 cm and 28.39 cm under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Plant Height

Source	DF	SS	MS	F	P
GENO	4	83.665	20.916	15255**	0
Treatment	3	505.84	168.613	122976**	0
GENO*treatment	12	0.077	0.006	4.68*	0.0003
Error	30	0.041	0.001		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Plant Height for Treatment

Treatment	Mean	Homogeneous Groups
3	34.152	A
2	31.147	B
1	27.227	C
0	25.184	D

LSD All-Pairwise Comparisons Test of Plant Height for GENO

GENO	Mean	Homogeneous Groups
1	31.503	A
2	30.432	B
3	29.399	C
4	28.392	D
5	27.411	E

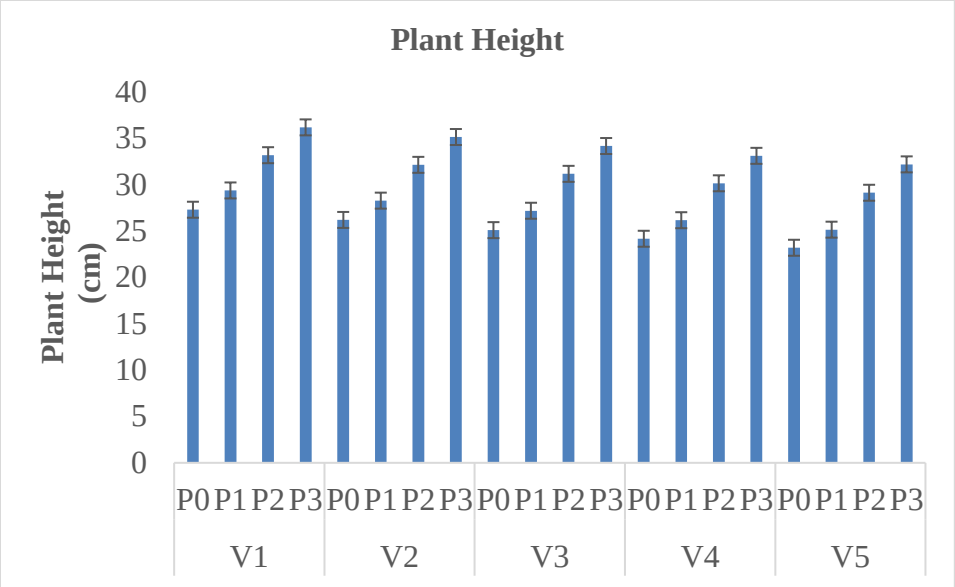


Fig.4.18: Graph showing Plant Height (cm) of Physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.2 Root Length

Root lengths of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the root length of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in root length. For example, the average root length of variety V1 (Swat 84) is 22.91 cm under control conditions (C0), but it increases to 25.95 cm under 1% concentration (C1), 29.99 cm under 2% concentration (C2) and 33.95 cm under 3% concentration (C3). On the other hand, the average root length of variety V5 (Malakand) is only 20.35 cm under control conditions and it only increases to 22.39 cm, 25.35 cm and 28.39 cm under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root Length

Source	DF	SS	MS	F	P
GENO	4	51.015	12.7538	459.61**	0
Treatment	3	78.9163	26.3054	947.98**	0
GENO*treatment	12	0.8684	0.0724	2.61*	0.0164
Error	30	0.8325	0.0277		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root Length for Treatment

Treatment	Mean	Homogeneous Groups
3	27.341	A
2	25.394	B
1	24.376	C
0	24.154	D

LSD All-Pairwise Comparisons Test of Root Length for Geno

GENO	Mean	Homogeneous Groups
1	26.977	A
2	26.162	B
3	25.182	C
4	24.32	D
5	23.941	E

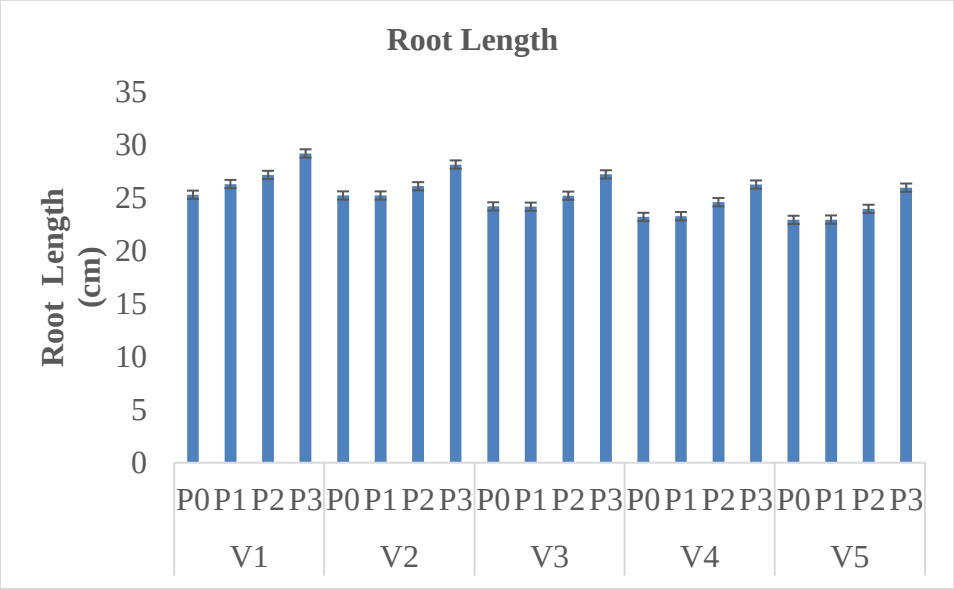


Fig.4.19: Graph showing Root Length (cm) of Physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.3 Shoot Length

Shoot lengths of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the shoot length of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in shoot length. For example, the average shoot length of variety V1 (Swat 84) is 25.32 cm under control conditions (C0), but it increases to 27.36 cm under 1% concentration (C1), 31.23 cm under 2% concentration (C2) and 34.36 cm under 3% concentration (C3). On the other hand, the average shoot length of variety V5 (Malakand) is only 20.35 cm under control conditions and it only increases to 22.39 cm, 25.35 cm and 28.39 cm under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Shoot Length

Source	DF	SS	MS	F	P
GENO	4	4.64	1.16	425.43**	0
Treatment	3	297.935	99.3116	36422.4**	0
GENO*treatment	12	1.63	0.1358	49.82**	0
Error	30	0.082	0.0027		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Shoot Length for Treatment

Treatment	Mean	Homogeneous Groups
3	31.882	A
2	29.905	B
1	26.578	C
0	25.176	D

LSD All-Pairwise Comparisons Test of Shoot Length for GENO

GENO	Mean	Homogeneous Groups
1	29.017	A
2	28.436	B
3	28.265	C
4	28.152	D
5	28.055	E

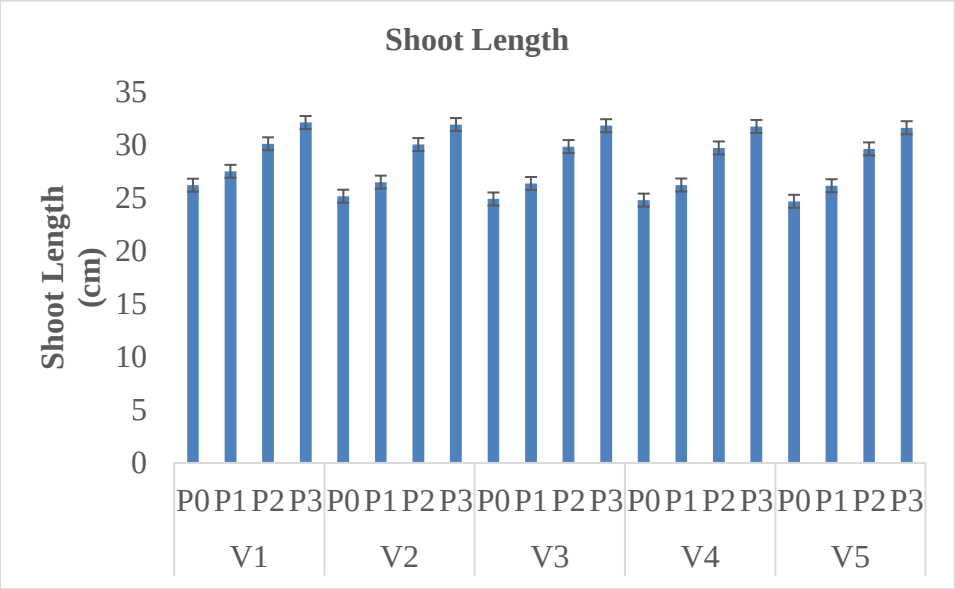


Fig.4.20: Graph showing Shoot Length (cm) of Physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.4 Root Fresh Weight

Root fresh weights of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the root fresh weight of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in root fresh weight. For example, the average root fresh weight of variety V1 (Swat 84) is 24.82 grams under control conditions (C0), but it increases to 28.16 grams under 1% concentration (C1), 29.69 grams under 2% concentration (C2) and 32.18 grams under

3% concentration (C3). On the other hand, the average root fresh weight of variety V5 (Malakand) is only 20.95 grams under control conditions and it only increases to 22.99 grams, 25.95 grams and 28.94 grams under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root Fresh Weight

Source	DF	SS	MS	F	P
GENO	4	0.06525	0.01631	13255.7**	0
Treatment	3	0.06844	0.02281	18538.4**	0
GENO*treatment	12	0.00407	3.40E-04	275.86**	0
Error	30	0.00004	1.23E-06		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root Fresh Weight for Treatment

Treatment	Mean	Homogeneous Groups
0	1.1012	A
2	1.0742	B
3	1.0379	C
1	0.9936	D

LSD All-Pairwise Comparisons Test of Root Fresh Weight for GENO

GENO	Mean	Homogeneous Groups
5	1.1163	A
4	1.0771	B
3	1.0422	C

1	1.0123	D
2	1.0106	E

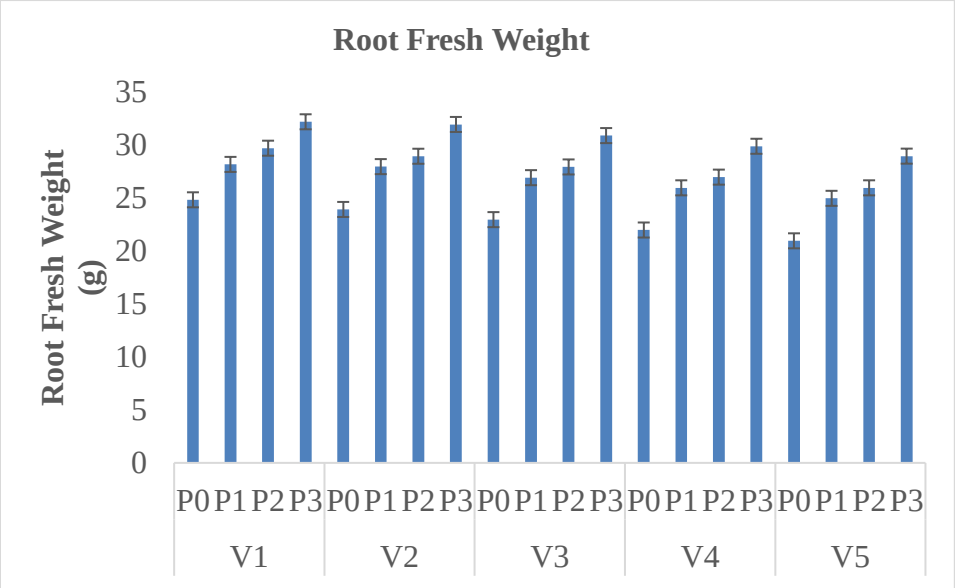


Fig.4.21: Graph showing Root Fresh Weight (g) of Physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.5 Root Dry Weight

Shoot dry weights of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the shoot dry weight of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in shoot dry weight. For example, the average shoot dry weight of variety V1 (Swat 84) is 4.38 grams under control conditions (C0), but it increases to 4.86 grams under 1% concentration (C1), 5.74 grams under 2% concentration (C2) and 6.62 grams under 3% concentration (C3). On the other hand, the average shoot dry weight of variety V5 (Malakand) is only 4.05 grams under control conditions and it only increases to 4.31 grams, 4.95 grams and 5.55

grams under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root Dry Weight

Source	DF	SS	MS	F	P
GENO	4	50.7114	12.6779	4711.01**	0
Treatment	3	44.5075	14.8358	5512.91**	0
GENO*treatment	12	4.8085	0.4007	148.9**	0
Error	30	0.0807	0.0027		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root Dry Weight for Treatment

Treatment	Mean	Homogeneous Groups
3	5.9567	A
2	4.9453	B
1	4.09	C
0	2.98	D

LSD All-Pairwise Comparisons Test of Root Dry Weight for GENO

GENO	Mean	Homogeneous Groups
1	6.1883	A
2	5.1917	B
3	4.4558	C
4	3.6292	D
5	3	E

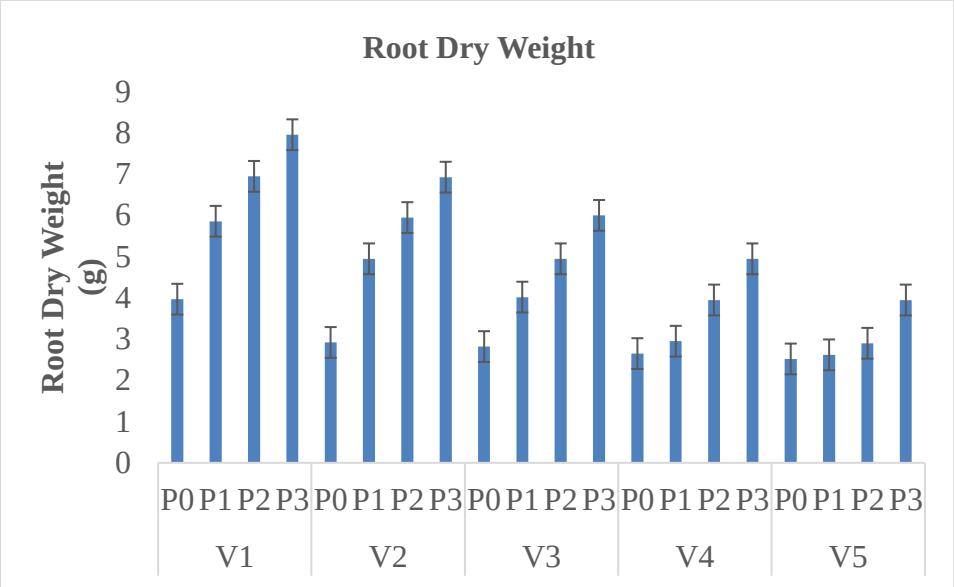


Fig.4.22: Graph showing Root Dry Weight (g) of physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.6 Shoot Fresh Weight

Shoot fresh weights of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the shoot fresh weight of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in shoot fresh weight. For example, the average shoot fresh weight of variety V1 (Swat 84) is 24.94 grams under control conditions (C0), but it increases to 26.95 grams under 1% concentration (C1), 28.92 grams under 2% concentration (C2) and 30.88 grams under 3% concentration (C3). On the other hand, the average shoot fresh weight of variety V5 (Malakand) is only 21.97 grams under control conditions and it only increases to 23.95 grams, 25.91 grams and 27.95 grams under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Shoot Fresh Weight

Source	DF	SS	MS	F	P
GENO	4	68.966	17.2416	8271.56**	0
Treatment	3	177.668	59.2227	28411.7**	0
GENO*treatment	12	1.151	0.0959	46**	0

Error	30	0.063	0.0021		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Shoot Fresh Weight for Treatment

Treatment	Mean	Homogeneous Groups
3	29.775	A
2	27.789	B
1	25.952	C
0	23.952	D

LSD All-Pairwise Comparisons Test of Shoot Fresh Weight for GENO

GENO	Mean	Homogeneous Groups
1	28.588	A
2	27.926	B
3	26.927	C
4	25.946	D
5	24.947	E

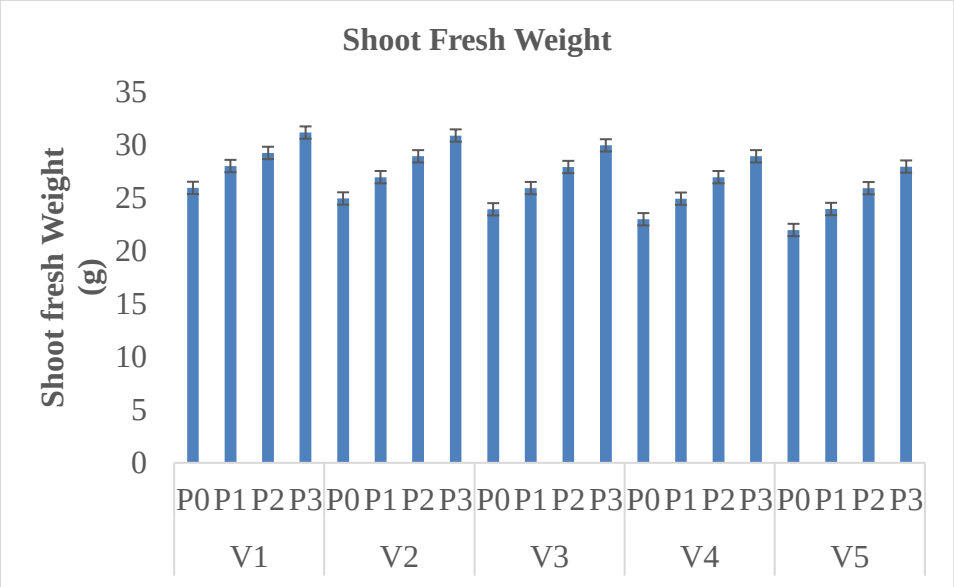


Fig.4.23: Graph showing Shoot Fresh Weight (g) of Physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.7 Shoot Dry Weight

Shoot dry weights of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the shoot dry weight of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in shoot dry weight. For example, the average shoot dry weight of variety V1 (Swat 84) is 4.38 grams under control conditions (C0), but it increases to 4.86 grams under 1% concentration (C1), 5.74 grams under 2% concentration (C2) and 6.62 grams under 3% concentration (C3). On the other hand, the average shoot dry weight of variety V5 (Malakand) is only 4.05 grams under control conditions and it only increases to 4.31 grams, 4.95 grams and 5.55 grams under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Shoot Dry Weight

Source	DF	SS	MS	F	P
GENO	4	60.7771	15.1943	2156.23**	0
Treatment	3	79.2315	26.4105	3747.94**	0
GENO*treatment	12	2.5726	0.2144	30.42**	0

Error	30	0.2114	0.007		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Shoot Dry Weight for Treatment

Treatment	Mean	Homogeneous Groups
3	6.9667	A
2	4.9933	B
1	4.0453	C
0	3.662	D

LSD All-Pairwise Comparisons Test of Shoot Dry Weight for GENO

GENO	Mean	Homogeneous Groups
1	6.715	A
2	5.7017	B
3	4.9667	C
4	3.9742	D
5	3.2267	E

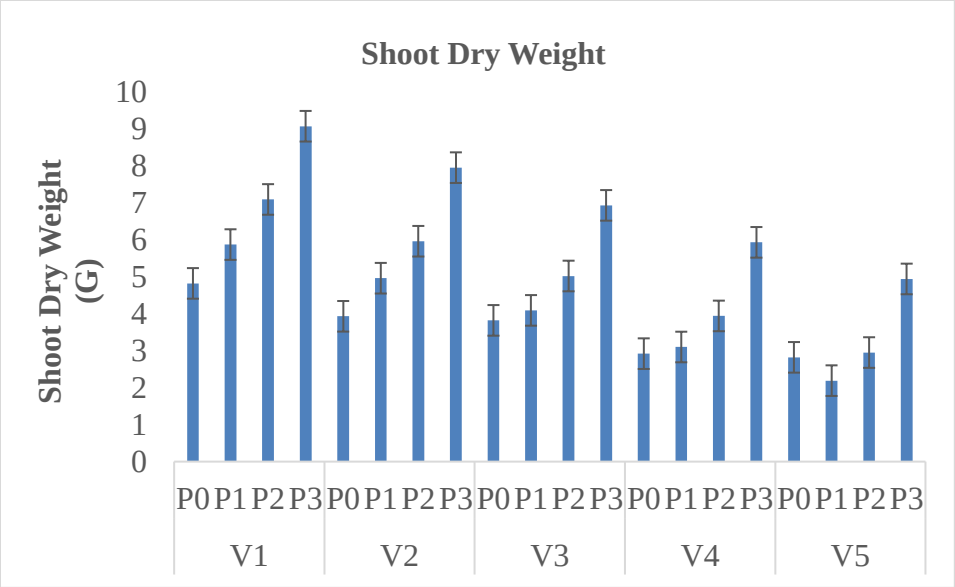


Fig.4.24: Graph showing Shoot Dry Weight (g) of physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.8 Germination %

Germination percentage of all five varieties of soybean increases with increasing concentration of fungicide. However, the increase is not uniform across all varieties. Swat 84 shows the greatest increase in germination percentage. For example, the average germination percentage of variety V1 (Swat 84) is 70% under control conditions (C0), but it increases to 80.22% under 1% concentration (C1), 85.16% under 2% concentration (C2) and 97.17% under 3% concentration (C3). On the other hand, the average germination percentage of variety V5 (Malakand) is only 63.33% under control conditions and it only increases to 78.14%, 83.17% and 94.18% under 1%, 2% and 3% concentrations, respectively. This suggests that variety V1 (Swat 84) is more responsive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Germination %

Source	DF	SS	MS	F	P
GENO	4	99.45	24.86	1138.56**	0

Treatment	3	3929.52	1309.84	59980.5**	0
GENO*treatment	12	15.52	1.29	59.21**	0
Error	30	0.66	0.02		
Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Germination % for Treatment

Treatment	Mean	Homogeneous Groups
3	95.192	A
2	83.943	B
1	78.978	C
0	65.712	D

LSD All-Pairwise Comparisons Test of Germination % for GENO

GENO	Mean	Homogeneous Groups
1	83.139	A
2	82.34	B
3	80.665	C
4	79.707	D
5	78.93	E

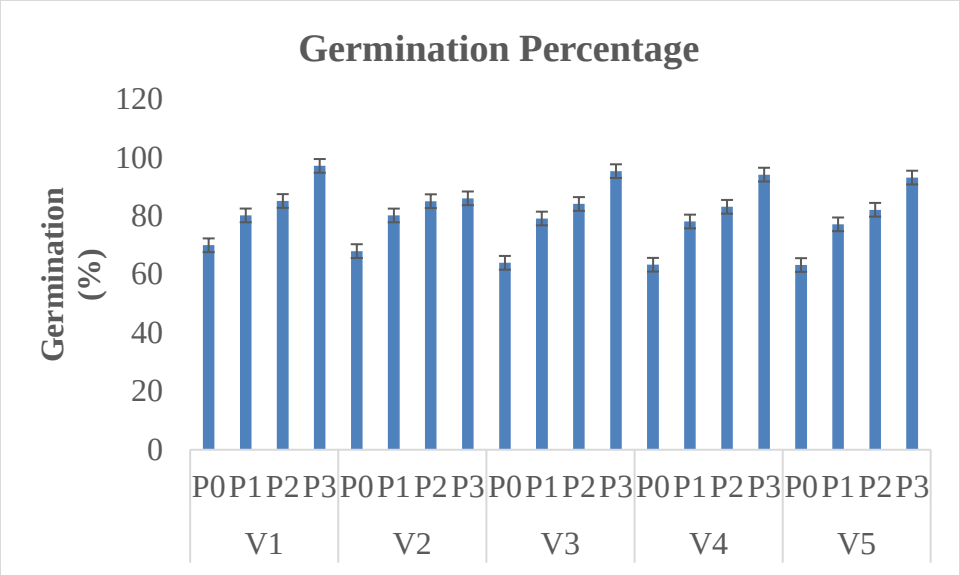


Fig.4.25: Graph showing germination (%) of physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.5.3.9 Root /Shoot Ratio

Root/shoot ratios of five different varieties of soybean (Swat 84, Faisal, Ajmeri, SL-80 and Malakand) under three different concentrations of fungicide (C0-C3). C0 is the control group, while C1, C2 and C3 represent 1%, 2% and 3% concentrations of fungicide, respectively. The table shows that the root/shoot ratio of all five varieties of soybean decreases with increasing concentration of fungicide. However, the decrease is not uniform across all varieties. Swat 84 shows the greatest decrease in root/shoot ratio. For example, the average root/shoot ratio of variety V1 (Swat 84) is 0.96 under control conditions (C0), but it decreases to 0.93 under 1% concentration (C1), 0.92 under 2% concentration (C2) and 0.88 under 3% concentration (C3). On the other hand, the average root/shoot ratio of variety V5 (Malakand) is 1.00079 under control conditions and it only decreases to 0.97 under 1% concentration, 0.95 under 2% concentration and 0.94 under 3% concentration. This suggests that variety V1 (Swat 84) is more sensitive to the fungicide than variety V5 (Malakand).

Analysis of Variance Table for Root /Shoot Ratio

Source	DF	SS	MS	F	P
GENO	4	65.679	16.4197	7208.63 **	0
Treatment	3	265.407	88.4689	38840 **	0
GENO*treatment	12	0.561	0.0467	20.52 **	0
Error	30	0.068	0.0023		

Total	49				

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Root /Shoot Ratio for Treatment

Treatment	Mean	Homogeneous Groups
3	30.762	A
2	27.895	B
1	26.787	C
0	22.92	D

LSD All-Pairwise Comparisons Test of Root /Shoot Ratio for GENO

GENO	Mean	Homogeneous Groups
1	28.714	A
2	28.182	B
3	27.167	C
4	26.191	D
5	25.202	E

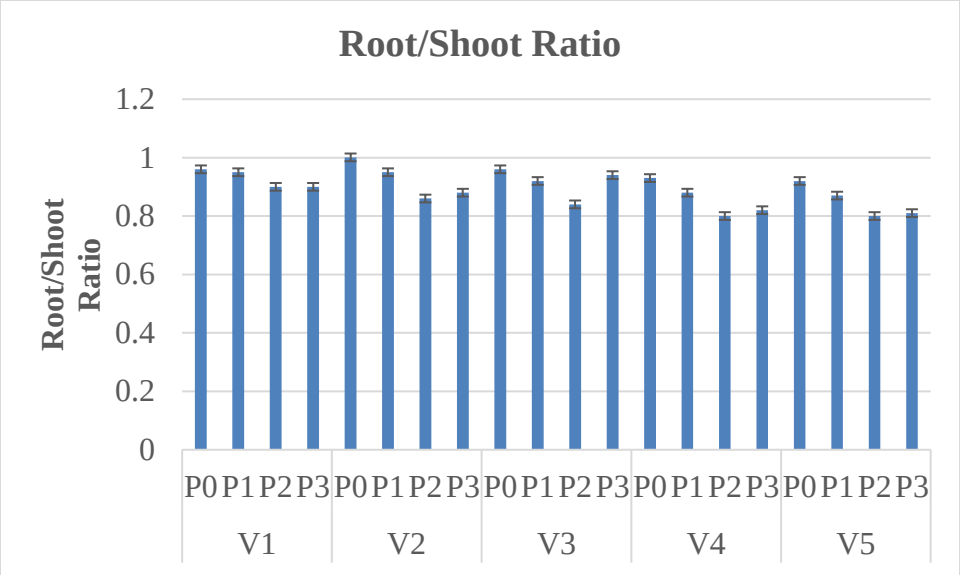


Fig.4.26: Graph showing Root/Shoot Ratio of physically treated soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Varieties and y-axis= Concentrations)

4.6 Disease incidence

The disease incidence of soya bean seeds was evaluated against the fungi (*Fusarium spp.*) as the proportion of infected plants over the total number of plants by the formula. The results were recorded for three replications after 24, 48 and 72 hours with concentrations of 1%, 2% and 3%. Based on the table, it is evident that several sources have low p-values (e.g., "TR," "CON," "DAYS"), indicating statistically significant differences among the group means. These significant differences suggest that the variables "TR," "CON," and "DAYS" have a significant impact on the dependent variable under study. However, for the interactions between the factors (e.g., "TR*CON*," "*TR*DAYS," "CON*DAYS*," "*TR*CON*DAYS"), the p-values are relatively high (greater than 0.05), suggesting that these interactions do not have a significant effect on the dependent variable. Overall, this ANOVA test provides valuable insights into the relationships between the variables and highlights which sources of variation have a significant impact on the outcome being studied.

Analysis of Variance Table for Disease Incidence

Source	DF	SS	MS	F	P
REP	2	36.6	18.3		
TR	1	11229.7	11229.7	2061.17**	0
CON	2	67.8	33.9	6.22*	0.005

DAYS	2	64	32	5.87*	0.0065
TR*CON	2	168.5	84.2	15.46**	0
TR*DAYS	2	677.9	338.9	62.21**	0
CON*DAYS	4	33.8	8.5	1.55ns	0.2092
TR*CON*DAYS	4	20.1	5	0.92ns	0.4614
Error	34	185.2	5.4		
Total	53	12483.6			

SOV= Source of variance, **DF**= Degree of freedom, **MS**= Mean square, **SS**=Sum of square

LSD All-Pairwise Comparisons Test of Disease Incidence for Concentration.

CON	Mean	Homogeneous	Groups
1	41.294	A	
2	39.929	AB	
3	38.55	B	

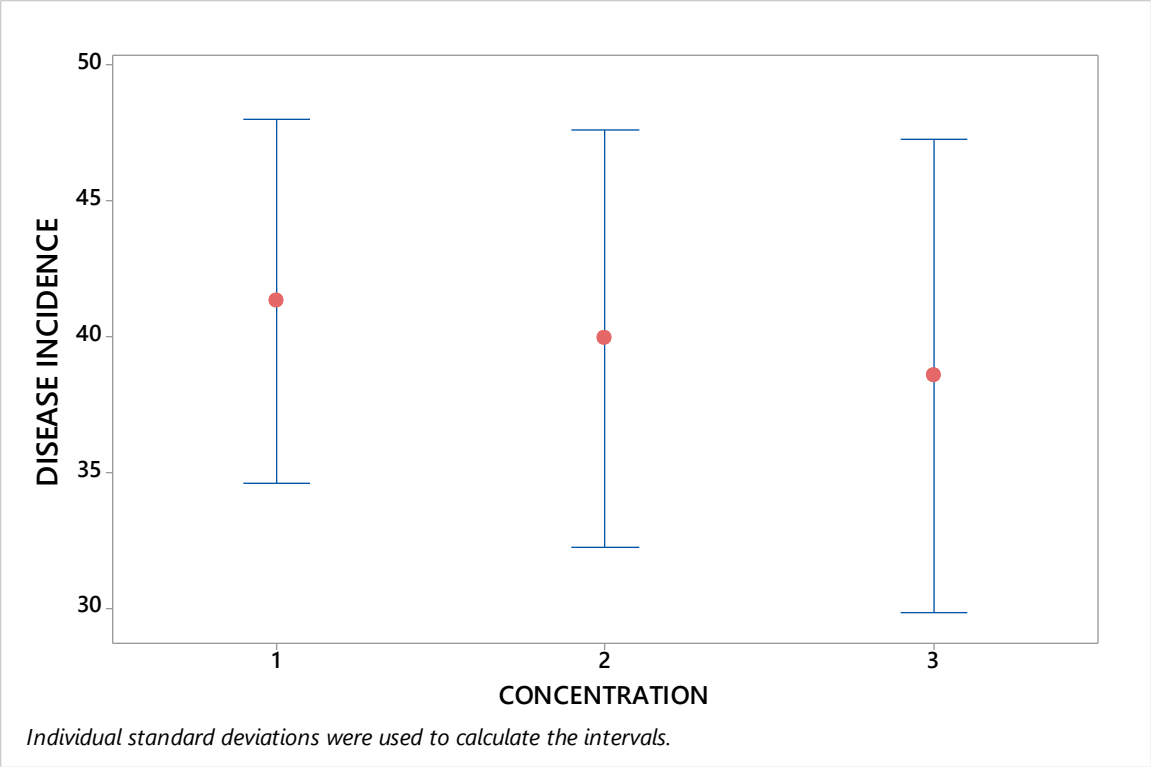


Fig.4.27: Graph showing Disease Incidence for concentration in soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Concentration and y-axis= Disease Incidence)

LSD All-Pairwise Comparisons Test of Disease Incidence for Days.

DAYS	Mean	Homogeneous	Groups
3	41.25	A	
2	39.939	AB	
1	38.584	B	

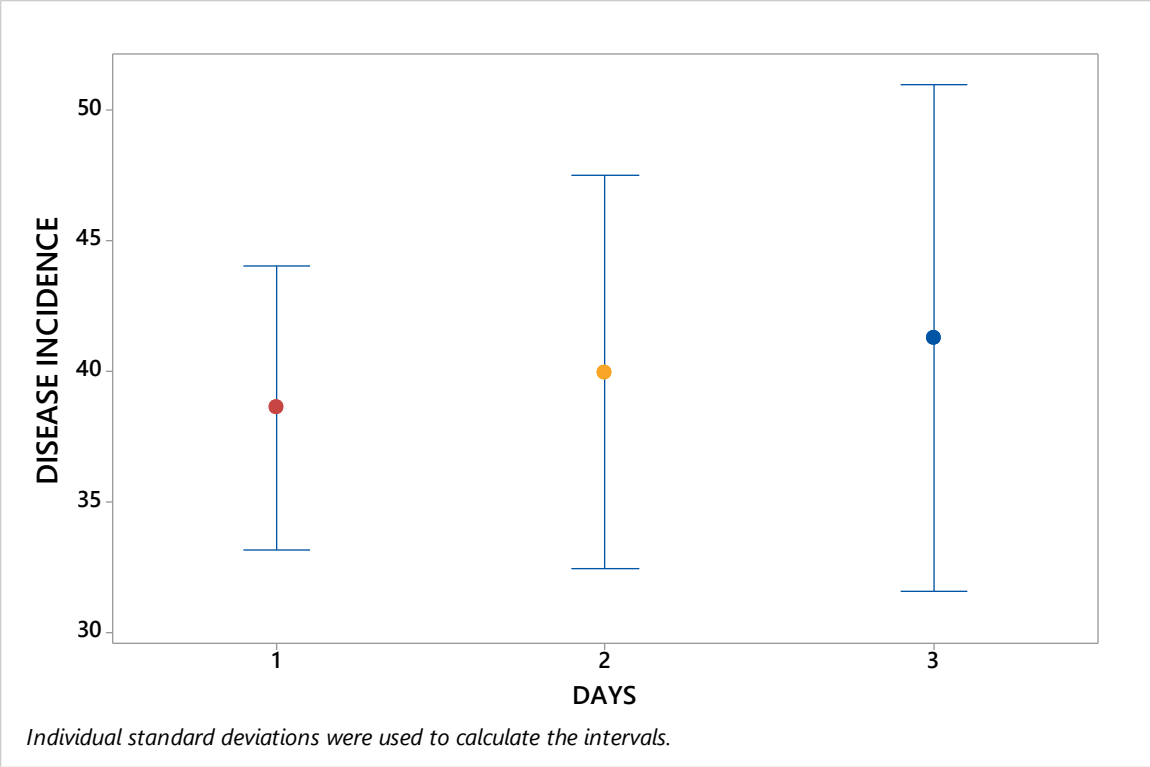


Fig.4.28: Graph showing Disease Incidence for days in soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Days and y-axis= Disease Incidence)

LSD All-Pairwise Comparisons Test of Disease Incidence for Treatment.

TREATMENT	Mean	Homogeneous	Groups
2	54.345	A	
1	25.504	B	

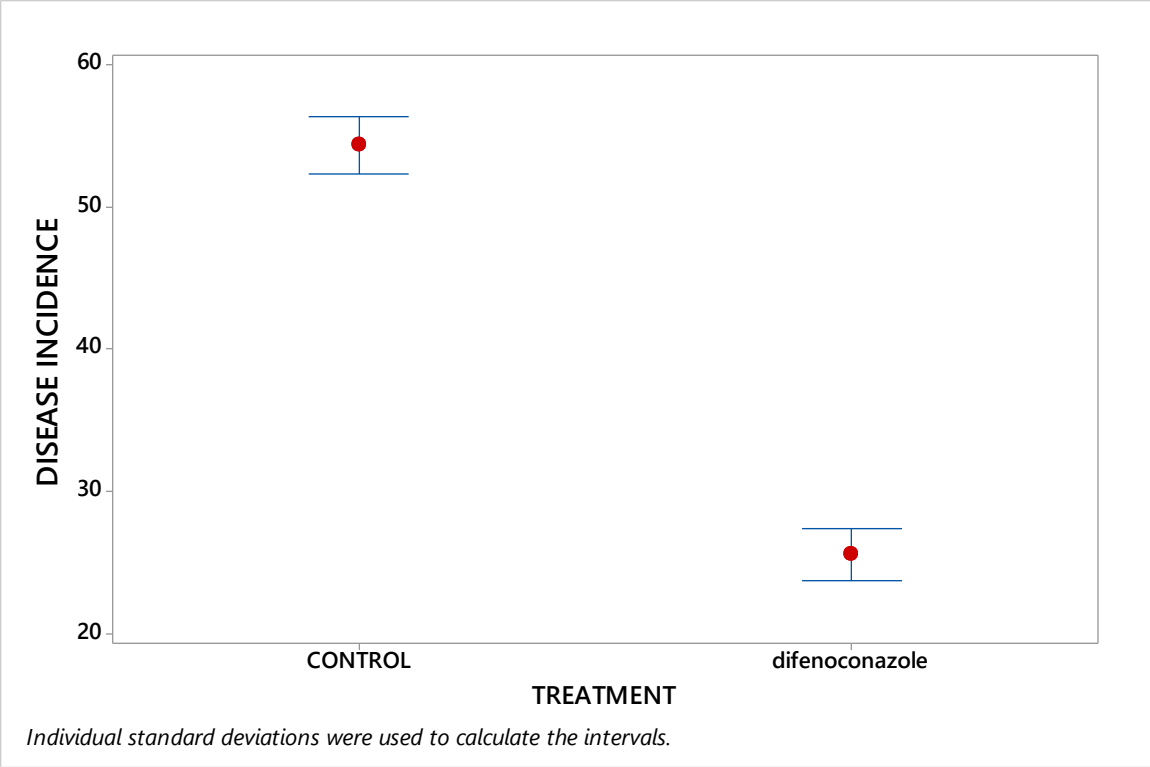


Fig.4.29: Graph showing Disease Incidence for treatment in soybean varieties i.e. V1= Swat 84, V2= Faisal, V3= Ajmeri, V4= SL 80, V5= Malakand (x-axis= Days and y-axis= Disease Incidence)

Discussion

A forty-day research was conducted at the Phytopathology Laboratory, UAF. Three fungal species were isolated from *Soybean*varieties and identified as ascomycetous fungi, where the most common genera across three regions were *Fusarium* and *Alternaria*. The identification results of current study, show that the fungi carried by soybean seeds are *Fusarium sp.*, *Alternaria sp.* and *Saprophyte sp*. (Pitt and Hocking, 2009) reported that these species are associated with several soybean diseases, *Fusarium* species are also associated with several plant diseases such as vascular wilts, root and stem rots among others. The use of fungicides as seed treatments has been reported to suppress pathogenic infections, particularly fungi, during 3-4 weeks of storage. The presence of pathogens in seeds causes a decrease in viability, a musty odor and a change in seed color. Antifungal activity of plant extracts was tested against two pathogenic fungi, *Fusarium* and *Alternaria spp.* isolated from *Soybean*using Poison Food Technique. All the samples tested were found effective in-vitro. More than 40-45 % inhibition of growth of individual fungal species was observed at 100 ppm. Maximum inhibition was observed at concentration of 300 ppm. However, (Bhuyan *et al.*, 2015) reported that *Alternaria spp.* exhibited the strongest activity 80%and 78.6% in the case of *Fusarium oxysporum* at concentration of 1000 ppm. Variance analysis and mean comparison results displayed that mean germination time and the time to get 100% germination were affected by different fungicide concentrations and seed priming duration. Germination percentage of all five varieties of soybean increases with increasing concentration of fungicide.

However, the increase is not uniform across all varieties. The highest germination index was observed in V1(SWAT 84) i.e.,72% while it has been reported in previous studies that primed seeds showed better germination pattern and higher vigor level than non-primed and 66% germination was reported in treated seeds (Sadhegi *et al.*, 2011). This study used treatments, namely physical therapy by heating the seeds in a microwave at a temperature of 40^{o}C for 10, 20 30 seconds and chemical treatment by soaking the seeds in a fungicide with active ingredient difenoconazole with a concentration of 1%, 2% and 3%. Seeds without heating and as control are soaking fungicides. Each treatment was repeated three times. The seeds that have been given treatments are then planted using the growing test technique and incubated for seven days. Physical and chemical treatments did not significantly affect seed viability. All treatments showed that the seed viability was up to 100%. Reported studies have shown that a successful seed treatment should reduce microbial pathogens while preserving seed viability and germination (95%), seed vigor and the sensorial attributes of the final product (FDA, 2017). In general, physical and chemical treatments can suppress the growth of fungi. Chemical treatment with a concentration of 1%, 2% and 3% significantly affected the *Fusarium*. Based on these results, it is suspected that the physical and chemical treatments do not interfere with plant physiological processes. In contrast, the report of (Ramdan *et al.*, 2020) stated that physical treatment has higher viability than the chemical treatment of corn seeds.

Suggestions for future research

Breeding programs designed to develop soybean varieties with built-in resistance to common pathogens could play a pivotal role in reducing reliance on chemical treatments. A comprehensive analysis of the soybean seed microbiome is essential to comprehend the intricate interactions between beneficial and pathogenic microorganisms. This holistic approach can guide the development of seed treatments that actively support and foster beneficial microbial communities. Moreover, it is imperative to assess the long-term impact of seed treatments on soil health, investigating whether residues from chemical treatments exert any adverse effects on beneficial soil microorganisms and overall soil ecology. Lastly, a thorough economic analysis is warranted to evaluate the cost-effectiveness of the recommended seed treatment approach in comparison to traditional methods. Considering economic feasibility will be instrumental in facilitating the practical implementation of the suggested treatments by farmers, ensuring their widespread adoption in agricultural practices.

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