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Evaluation of varietal resistance and efficacy of botanical and chemical treatments against seedborne fungal pathogens in safflower (*Carthamus tinctorius* L.)

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Abstract

Safflower (*Carthamus tinctorius* L.) is a valuable oilseed crop cultivated for its economic and nutritional benefits; however, its productivity is often compromised by seed-borne fungal infections. Despite growing interest in environmentally friendly plant protection strategies, limited research has addressed the comparative impact of multiple fungal pathogens on different safflower varieties and the integrated use of botanical and chemical control methods. This study aims to investigate the incidence and pathogenic effects of five seed-borne fungi *Alternaria carthami*, *Aspergillus niger*, *Rhizopus* sp., *Curvularia* sp., and *Fusarium oxysporum* isolated from four safflower varieties (S-208, Thori-78, SAF-130, and SAF-30), and to evaluate the efficacy of selected plant extracts and commercial fungicides in controlling fungal growth. Results revealed *A. niger* as the most prevalent pathogen, with isolation rates between 27.50% and 61.50%, followed by *A. carthami* (23.00%–40.00%). The Thori-78 variety showed the highest incidence of fungal contamination, while S-208 showed the lowest. *Rhizopus* sp. was not isolated from S-208. Infected seeds demonstrated significantly lower germination rates, with the healthiest germination observed in non-infected S-208 seeds. *In vitro* antifungal tests with plant extracts *Aloe vera*, toothbrush plant, garlic, neem, and giant milkweed showed garlic extract as the most effective at all tested concentrations (10%, 20%, 30%), followed by neem. Among five fungicides (Aliette, Topsin-M, Dithane M-45, Carbendazim and Acrobat), Dithane M-45, Carbendazim, and Topsin-M showed the highest antifungal activity, especially at higher doses, and promoted improved plant height and biomass. This study fills a key research gap by combining varietal susceptibility analysis with integrated fungal management strategies, highlighting the potential of both botanical and synthetic agents in safeguarding safflower crops and enhancing overall agricultural productivity.

Keywords: Botanical extracts, Fungicides, Management, Safflower, Seedborne fungi, Varieties

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Introduction

The annual oilseed crop known as safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae (Compositae) family. This family is well-known and renowned as the largest family of flowering plants that has 22,000 species and over 1,500 genera, ranging from woody shrubs to annual herbs. Among all species, *C. tinctorius* is the only species of this genus cultivated for edible oil and other benefits for humans (Berville et al., 2005; Griffee, 2000). Initially, safflower was cultivated for its flowers, which contained beautiful colors used for making dyes and paints. It is still utilized in paints and varnishes due to its characteristics of non-yellowing. Today, safflower is a favored food in the bird-seed industry, particularly for species in the parrot family and pigeons, as well as for producing edible seed oil, which is used in cooking, salad oils, and margarine (Mündel et al., 2004; Singh and Nimbkar, 2006). Cultivated safflower varieties are rich in oil, containing nearly 30-40% edible oil, 20% protein, and 35% fiber (Sehgal & Raina, 2011).

Various biotic and abiotic factors have been reported by different groups to influence crop production technology (Zaman & Qureshi, 2018; Shah et al., 2019; Iqbal & Qureshi, 2021; Zia et al., 2023). Fungi, bacteria, and viruses are among the biotic agents that become more active and significantly reduce cultivated crops, especially under favorable conditions for infection development (Singh & Nimbkar, 2006; Ahmad & Ahmad, 2018; Rubab et al., 2020; Zafarullah et al., 2021; Hameed et al., 2022). Safflower cultivation is significantly affected by seedborne fungi, which impair seed germination, reduce plant vigor, and deteriorate both the quality and quantity of the produce; additionally, poor-quality seed and associated pathogens are major factors contributing to poor

crop stand and yield loss (Ramesh & Avitha, 2005; Gayathri et al., 2014). Safflower is an important oilseed crop, increasingly threatened by seedborne fungal pathogens that significantly impair germination, reduce plant vigor, and lower both yield and quality of the produce. Poor seed quality, often exacerbated by pathogenic contamination, remains one of the primary reasons for reduced crop stand and productivity in safflower cultivation (Gayathri et al., 2014). These seedborne fungi not only compromise agricultural output but also pose serious health and food safety risks due to their ability to produce harmful mycotoxins, which can adversely affect both humans and animals (Makun et al., 2010). Numerous fungal species have been implicated in the degradation of safflower seeds. Studies have identified species such as *Aspergillus niger, A. flavus, Alternaria dianthicola, Fusarium oxysporum, F. equiseti, Macrophomina phaseolina, Penicillium digitatum,* and *Rhizopus stolonifer,* all of which contribute to seed browning, rotting, necrosis, germination failure, and oilseed toxicity (Chavan & Kakde, 2008). The widespread incidence and diversity of seedborne fungi in various safflower cultivars including Bhima, HUS-305, A-300, S-144, K-1, and others have been documented, with significant reductions observed in seed germination and seedling vigor (Raghuwanshi & Deokar, 2002; Shaker, 2016; Awadhiya, 1992).

Beyond safflower, similar fungal pathogens have also been reported in a range of vegetable crops, suggesting a broader environmental prevalence and adaptive capacity of these fungi (Hamim et al., 2014; Qasim et al., 2023; Ullah et al., 2023). This highlights the urgent need for integrated disease management approaches that can minimize seedborne infections in an eco-friendly and sustainable manner. One promising avenue involves the use of plant-derived antifungal agents. Several medicinal plants have shown strong antifungal properties in vitro, offering an alternative to synthetic fungicides. For instance, extract from *Azadirachta indica* (neem), have demonstrated effectiveness against key pathogens such as *Aspergillus* spp., *Candida albicans,* and *Trichophyton rubrum* (Patil et al., 2017; Mangi et al., 2019; Mangi et al., 2021). Other plants like *Aloe vera* have also shown notable antifungal activity, especially against *Aspergillus niger* and *Fusarium* spp. (Saniasiaya et al., 2017; Hasan et al., 2005; Mahmoud et al., 2011).

Fungicides are widely recommended as an effective strategy for controlling *Aspergillus niger* and related pathogenic fungi. In particular, active ingredients such as Copper Hydroxide (77%), Mancozeb (75%), and Carbendazim (50%) have been evaluated for their efficacy against *Aspergillus flavus*, a closely related and economically significant fungal pathogen. Studies have shown that Carbendazim and Mancozeb significantly suppress mycelial development and sclerotia germination compared to Copper Hydroxide. Furthermore, while Carbendazim and Copper Hydroxide were found to inhibit the production of aflatoxin B1, Mancozeb did not contribute to increased aflatoxin levels (Nayak et al., 2018). Additional research supports the effectiveness of various commercially available fungicides such as Difenoconazole, Miconazole, Fenpropimorph, Tridemorph, Triadimenol, and others against the vegetative growth, spore germination, and toxin production of *A. flavus* and other fungi (Aggarwal et al., 2005; Sarita et al., 2014).

Moreover, fungicides like Tecto, Benlate, Bayton, Topsin, and Derosal have demonstrated considerable success in eliminating seed-borne pathogens in sunflower cultivars HO-1 and NK-212 while improving seed germination (Rauf Bhutta et al., 2001). Similarly, Benomyl has shown superior effectiveness against a range of fungal species, including *Rhizoctonia solani, Fusarium oxysporum, Drechslera specifera, Macrophomina phaseolina, Alternaria alternata,* and *Cladosporium cladosporioides* (Nasir, 2003). In rice, seed dressing fungicides such as Thiovit, Cupravit, and Vitavax 200 (each at 0.25%) have significantly reduced infections by seedborne pathogens like *Bipolaris oryzae, Alternaria padwickii, Fusarium moniliforme, F. oxysporum, Aspergillus* spp., and *Curvularia lunata*, thereby improving seed quality and germination rates (Naher et al., 2016).

Materials and Methods

The study was conducted at the Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, where four safflower seed varieties including; S-208, Thori-78, SAF-130, and SAF-30 were collected from the Oilseeds Section, Agriculture Research Institute, Tandojam, for the isolation of seedborne fungi associated with safflower.

Isolation of seedborne fungi from safflower varieties

For each cultivar, two samples (100 seeds each sample) were chosen at random. To get rid of the saprophytic organisms on the seed surface, each sample was dipped in 0.01% mercuric chloride for two to three minutes and then rinsed in two changes of distilled water for three to four minutes each. Five seeds that were treated were placed in each petri plate comprising potato dextrose agar media that has been sterilized. Every petridish was kept at room temperature. Regular observations were made to monitor the development of fungus linked to safflower seeds. The following formula was used to record the incidence percentage of each fungus with the seed:

$$\text{Incidence Percent} = \frac{No.of\ seeds\ colonizes\ in\ each\ plate\ by\ a\ particular\ species}{Total\ number\ of\ seeds\ in\ each\ petridish} \times 100$$

Impact of seedborne fungi on seed germination of safflower varieties

Safflower healthy and diseased (symptomatic) seeds were isolated from the seed lot. A total of 200 seeds from every variety were chosen. Each petri plate, which had three layers of moistened blotter paper, included five seeds. Every plate was incubated at room temperature, and the germination of seeds from both healthy and infected seeds of several safflower was routinely observed.

Preparation of stock solutions

Fresh and healthy plant parts were collected from local markets and agricultural fields in the vicinity of Sindh Agriculture University, Tandojam, such as the leaves and bulbs of *Aloe vera*, neem, tooth brush plant, garlic, and giant milk weed. These were ground into a powder after being carefully cleaned with tap water and dried on blotting paper. Distilled water was used to soak the powdered plant materials for a full day at room temperature. Subsequent solutions were filtered via through muslin cloth.

Effect of different botanicals on the colony growth of *Aspergillus niger*

Three concentrations 10, 20, and 30% were created for this experiment by adding the proper amount of distilled water to the stock solution. PDA medium was added along with extracts at varying quantities. To prevent bacterial contamination, 250 milliliters of medium were supplemented with one antibacterial Chloromycetin capsule. Mycelial discs from the fungus's actively expanding edges were aseptically moved to the middle of each petri plate with the aid of a sterilized cork borer. The control group consisted of the untreated inoculation petridishes. Petri plates were incubated at 27 ± 2°C until the fungus's mycelial growth completely covered the control plates. Radial colony growth statistics were measured in millimeters, and the treatments' percentage of inhibition was computed. The following formula was used to determine the percentage of radial mycelial growth inhibition above control:

$$I = \frac{(C - T)}{C} \times 100$$

Where "I" represents the percent inhibition, C denotes the radial colony growth in the control group, and T indicates the radial colony growth in the treatment group.

Impact of several fungicides on the development of *Aspergillus niger*

Five distinct fungicides, including Aliette, Topsin-M, Dithane M-45, Carbendazim and Acrobat, were evaluated at various concentrations to assess their impact on the fungus. Three concentrations (1000, 1500, and 2000 ppm) of each fungicide were evaluated utilizing the poisoned food technique. (Nene & Thapaliyal, 1993). The potato dextrose agar (PDA) medium devoid of fungicide functioned as the control. Agar discs sourced from the actively proliferating margins of *A. carthami* cultures, utilizing a sterilized cork borer (6 mm diameter), were positioned at the center of each petri plate filled with sterilized PDA medium. All petridishes were incubated at 27 ± 2 °C until the control plates were completely covered with the mycelial growth of the fungus. Each treatment was replicated four times. Measurements of radial mycelial growth (mm) were documented and subjected to statistical analysis to evaluate the differences among the various treatments. The efficacy percentage of various fungicides against fungal growth was determined using the formula:

$$\text{Efficacy } (\%) = \frac{(C - T)}{C} \times 100$$

Where, C = growth in control and T = growth in treatment

In this context, C represents the growth observed in the control group, while T denotes the growth seen in the treatment group.

Effect of different plant extracts on plant growth of safflower

The experiment was conducted using earthen pots with a diameter of 9 inches. All pots were thoroughly washed, dried, and subsequently sprayed with alcohol to prevent any saprophytic contamination. Aqueous extracts from various plant species listed in (Table 1) were prepared using the aforementioned method and administered at the following doses: D1 equals 10 ml, D2 equals 20 ml, and D3 equals 30 ml per pot containing sterilized soil. Untreated, inoculated pots (C1) and untreated, uninoculated pots (C2) were utilized as controls. The pots were organized using a randomized complete block design, incorporating three replications. Each pot received one petridish fully covered with *A. niger* growth and was left for seven days to allow the fungus to establish prior to the addition of the respective doses of plant extracts. Subsequently, all doses were meticulously combined with sterilized soil. Ten seeds of uniform size from the Thori-78 safflower variety were sown per pot and subjected to regular watering. Mortality percentage and growth parameters (shoot and root) were recorded after 45 days of sowing.

Table 1 List of different botanicals used in experiment

S. No.	English Name	Local Name	Scientific Name	Family	Plant part used
1	*Aloe vera*	Kunwar boti	*Aloe vera*	Liliaceae	Leaves
2	Neem	Nim	*Azadirachta indica*	Meliaceae	Leaves
3	Garlic	Thoom	*Allium sativum*	Liliaceae	Bulb
4	Giant milk weed	Akk	*Calotropis gigantae*	Asclepiadaceae	Leaves
5	Tooth brush plant	Khabar	*Salvadora presica*	Salvadoraceae	Leaves

Effect of different fungicides on plant growth of safflower

A pot experiment was performed to assess the effectiveness of commercial fungicides, specifically Acrobat, Topsin-M, Dithane M-45, Carbendazim, and Aliette, applied as a soil drench against *A. niger*. The applied doses were 2 g L-1, 3 g L-1, and 4 g L-1. Each fungicide was applied at a rate of 10 ml per pot, drenched into pots containing sterilized and infested soil. Control conditions were established using untreated inoculated and untreated uninoculated pots. The procedures for sowing seed, inoculating the fungus, designing the experiment, and observing data were consistent with the previously outlined methods.

Statistical analysis

Data were subjected to statistical analysis utilizing the 'student edition of Statistix, version 1.0' software. Analysis of variance and least significant difference (LSD) tests were conducted at a 5% significance level to assess differences among treatment means.

Results

Isolation of seedborne fungi from safflower varieties

Total five different fungi were identified such as; *Aspergillus niger, Alternaria carthami, Rhizopus* sp., *Curvularia* sp. and *Fusarium oxysporum*. *Aspergillus niger* (Fig. 1) was isolated as predominant fungus (27.50-61.50%) followed by *Alternaria carthami* (23.00-40.00%), *Fusarium oxysporum* (4.00-26.00%), *Curvularia sp*. (5.00-19.50%) and *Rhizopus* sp. (5.00-7.50%) respectively (Table 2). Overall Thori-78 variety showed higher percentage of seedborne incidence whereas; minimum percentage of incidence was seen in S-208 safflower variety. All five fungi were isolated from all four tested varieties excluding S-208 from which *Rhizopus* sp. was not noticed.

Impact of seedborne fungus on safflower variety seed germination

The germination % of both healthy and infected safflower seeds cultivated on moistened blotter paper revealed that the infected seeds germination rate was lower. Healthy S-208 seeds showed the highest rate of germination (90.50%), followed by SAF-130 (89.00%), whereas infected Thori-78 variety seeds showed the lowest rate of germination (42.50%) (Table 3).

Assessment of several plant extracts in relation to the mycelial development of *Aspergillus niger*

To assess the in vitro antifungal activity of various plant extracts against *A. niger,* fresh and healthy plant materials specifically the leaf and bulb of *Aloe vera*, toothbrush plant, garlic, neem, and giant milkweed were tested at three concentrations (10%, 20%, and 30%). The extracts were evaluated based on their ability to inhibit mycelial growth of the fungus. The degree of mycelial inhibition varied significantly among the different plant extracts and concentrations. Garlic extract exhibited the strongest antifungal activity, reducing mycelial growth to 14.25–22.50 mm across all concentrations (Fig. 2), followed by neem extract, which reduced growth to 17.00–25.50 mm. *Aloe vera* and Tooth brush plant showed moderate inhibition, while giant milk weed demonstrated the lowest antifungal efficacy, with mycelial growth ranging from 49.00 to 55.00 mm. Interestingly, all plant extracts were more effective at the lowest concentration (10%), with efficacy decreasing at higher concentrations. A similar trend was observed when comparing mean values across all concentrations (Table 4). Among all treatments, garlic and neem extracts consistently demonstrated the highest antifungal effectiveness, including in comparison to the control.

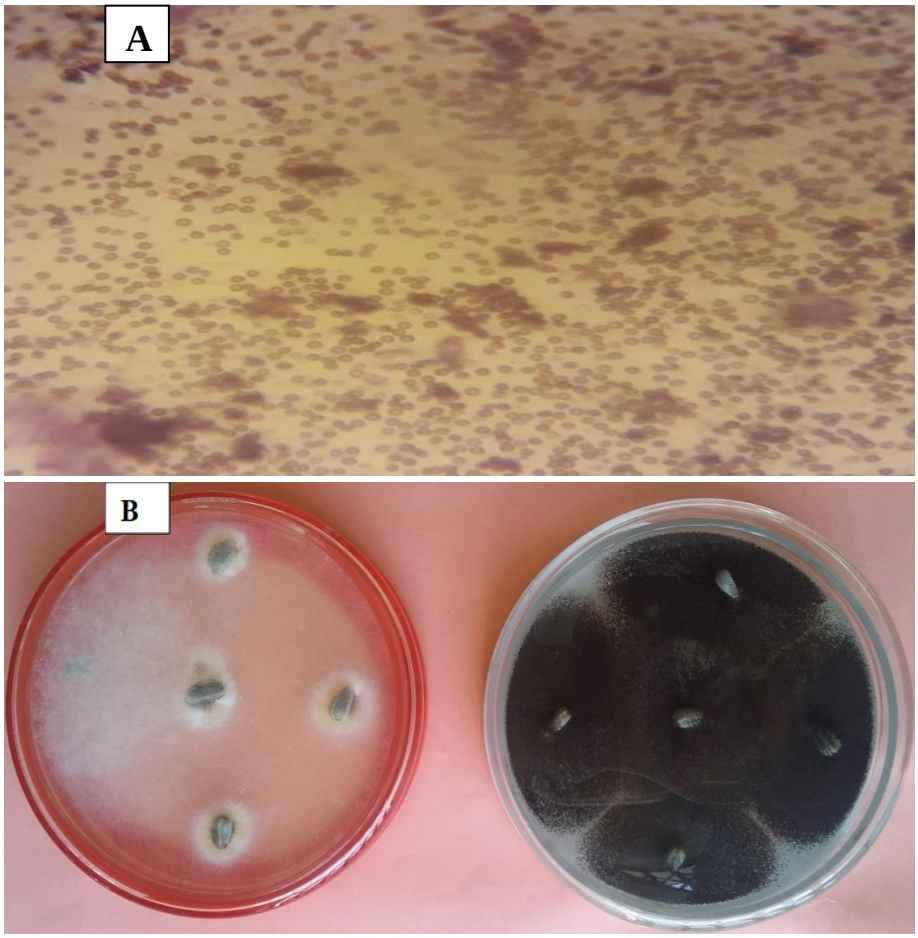

Fig. 1 (A) Spore of *Aspergillus niger* (B) Colony growth of *Aspergillus niger*

Table 2 Frequency of seedborne fungi associated with safflower varieties

Variety	Seedborne fungi	Number of infected seeds/200	Percentage
Thori-78	*Aspergillus niger*	123	61.50
	Alternaria carthami	80	40.00
	Curvularia sp.	32	16.00
	Fusarium oxysporum	20	10.00
	Rhizopus sp.	15	7.50
SAF-30	*Aspergillus niger*	111	55.50
	Alternaria carthami	65	32.50
	Curvularia sp.	23	11.50
	Fusarium oxysporum	52	26.00
	Rhizopus sp.	13	6.50
SAF-130	*Aspergillus niger*	99	49.50
	Alternaria carthami	58	29.00
	Curvularia sp.	39	19.50
	Fusarium oxysporum	42	21.00
	Rhizopus sp.	10	5.00
S-208	*Aspergillus niger*	55	27.50
	Alternaria carthami	46	23.00
	Curvularia sp.	10	5.00
	Fusarium oxysporum	08	4.00

Table 3 Germination percentage of healthy and infected seeds of safflower varieties

Variety	Type of seed	Number of seeds germinated/200	Percentage
Thori-78	Healthy	170	85.00
	Infected	85	42.50
SAF-30	Healthy	174	87.00
	Infected	87	43.50
SAF-130	Healthy	178	89.00
	Infected	90	45.00
S-208	Healthy	181	90.50
	Infected	95	47.50

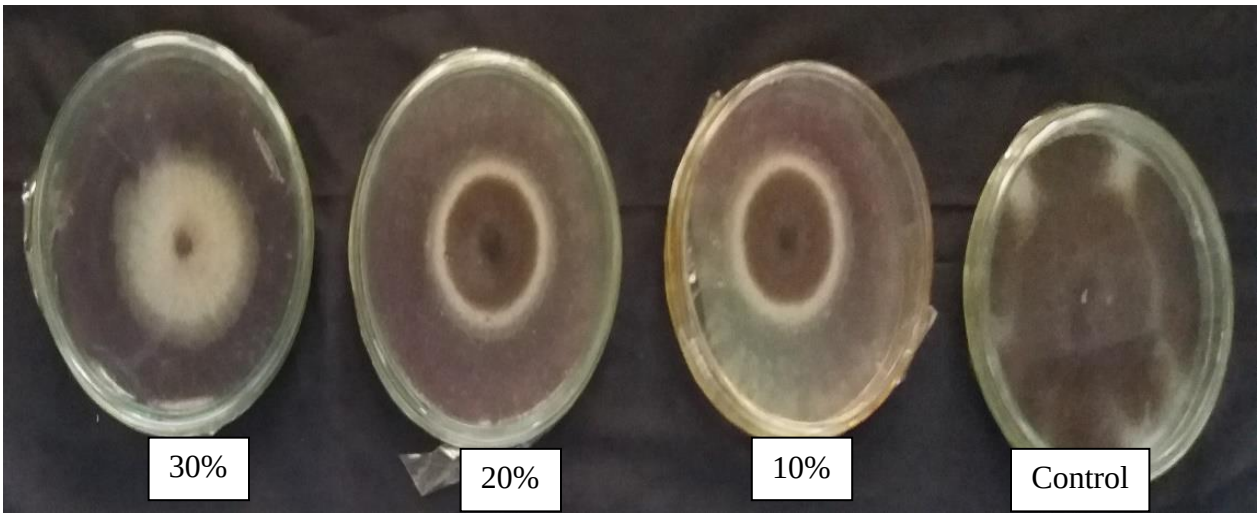

Fig. 2 Effect of garlic concentrations on mycelial growth of *Aspergillus niger*.

Table 4 Evaluation of different plant extracts against mycelial growth of *Aspergillus niger*

Plant extracts	Mycelial growth (mm) at different concentrations				
	10%	20%	30%	Mean	Inhibition (%)
Garlic	22.50^{f}	18.25^{f}	14.25^{f}	18.33^{e}	79.63
Neem	25.50^{e}	23.00^{e}	17.00^{e}	21.83^{cde}	75.74
Aloe vera	31.25^{d}	27.75^{d}	23.00^{d}	27.33^{cd}	69.63
Toothbrush plant	40.50^{c}	37.75^{c}	35.00^{c}	37.75^{c}	58.06
Giant milk weed	55.00^{b}	52.50^{b}	49.00^{b}	52.17^{b}	42.03
Control	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	-------
LSD (P=0.05)	1.441	1.371	1.693	6.115	

The alphabetical letters showing the homogenous grouping in column are not significant with each other.

Impact of various fungicides on *Aspergillus niger* colony growth

Effect of Fungicides on Mycelial Growth of *A. niger* (Tables 5 and 6) Fungicides showed significant variation ($P \leq 0.05$) in inhibiting mycelial growth of *A. niger* depending on both the type of fungicide and its concentration (Table 5). (Fig. 3) Dithane M-45 was the most effective fungicide at all three concentrations, reducing fungal growth to 3.00 mm at 1000 ppm, 2.00 mm at 1500 ppm, and 1.00 mm at 2000 ppm. Carbendazim followed, showing strong inhibition (5.00 mm to 2.00 mm) as the concentration increased. Topsin M also performed well but to a lesser extent, with growth ranging from 8.00 mm to 3.00 mm. Allitte and Acrobat were less effective, with Acrobat exhibiting the poorest inhibition (22.00, 29.00 mm) across all concentrations. The control treatment exhibited full radial growth (90.00 mm) in all cases. According to Table 6, the mean mycelial growth across all concentrations reinforced these findings: Dithane M-45 achieved 97.78% inhibition, followed by Carbendazim (95.92%) and Topsin M (93.70%). Allitte showed moderate inhibition (84.08%), while Acrobat was significantly less effective (71.48% inhibition). The Least Significant Difference (LSD) at P = 0.05 was 4.317, confirming that the differences among the top three fungicides (Dithane M-45, Carbendazim, and Topsin M) were statistically significant compared to less effective treatments.

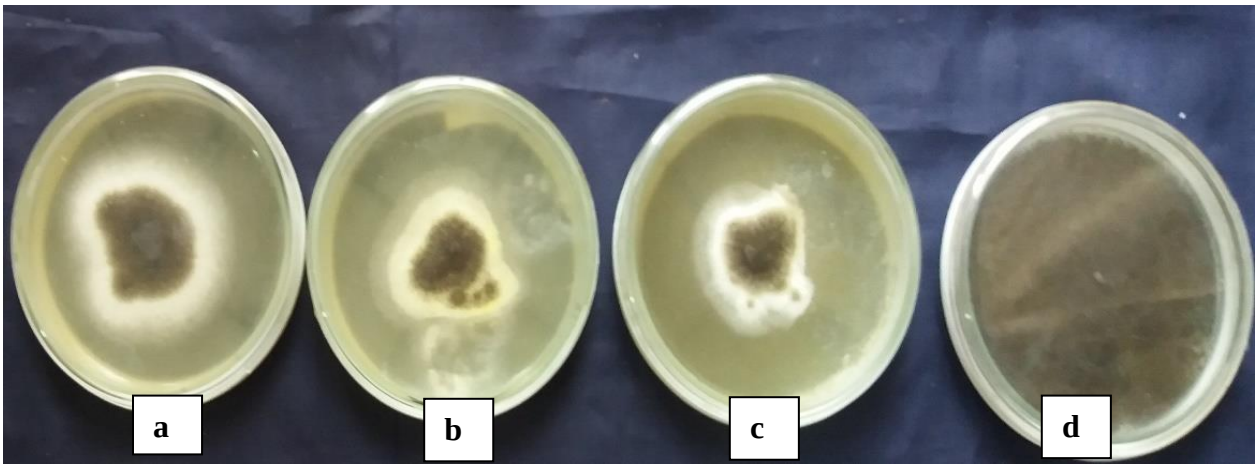

Fig. 3 Effect of Dithane M-45 on mycelial growth of *Aspergillus niger*. a) Dose 1; b) Dose 2; c) Dose 3; d) Control

Table 5 Effect of different fungicides on the colony growth of *Aspergillus niger*

Fungicides	Mycelial growth (mm) of the fungus at different concentrations		
	1000 ppm	1500 ppm	2000 ppm
Dithane M-45	3.00[f]	2.00[f]	1.00[e]
Carbendazim	5.00[e]	4.00[e]	2.00d[e]
Topsin M	8.00[d]	6.00[d]	3.00[d]
Allitte	18.00[c]	14.00[c]	11.00[c]
Acrobat	29.00[b]	26.00[b]	22.00[b]
Control	90.00[a]	90.00[a]	90.00[a]
LSD (P=0.05)	1.779	1.179	1.624

The alphabetical letters showing the homogenous grouping in column are not significant with each other.

Table 6 Mean colony growth at all concentrations of fungicides

Fungicides	Mycelial growth(mm)	Inhibition (%) over control
Dithane M-45	2.00[d]	97.78
Carbendazim	3.67[d]	95.92
Topsin M	5.67[d]	93.70
Allitte	14.33[c]	84.08
Acrobat	25.67[b]	71.48
Control	90.00[a]	------
LSD (P=0.05)	4.317	

The alphabetical letters showing the homogenous grouping in column are not significant with each other.

Impact of several plant extracts on safflower plant development

The study assessed the influence of various plant extracts on the growth and development of safflower plants inoculated with *A. niger*. The results revealed statistically significant differences ($P < 0.05$) among treatments in both plant height and plant biomass across three developmental stages (D1, D2, and D3), as shown in Table 7 (Fig. 4). The non-inoculated, non-treated control (Control-2) exhibited the greatest plant length and weight, consistently recording 65.00 cm plant height and 31.00 g plant weight at all stages. This confirmed the detrimental effect of A. niger on plant development when untreated. Among the extract treatments, garlic extract significantly enhanced plant growth under fungal stress. Plant length increased from 46.00 cm (D1) to 50.00 cm (D3). Plant weight ranged from 22.00 g to 25.45 g, placing garlic as the most effective among the botanical treatments. Neem extract was the second most effective treatment. Plant height ranged from 42.33 cm to 48.47 cm, and weight from 19.00 g to 22.00 g. *Aloe vera* showed a moderate effect with plant length ranging between 40.00–45.00 cm and plant weight from 17.00–20.00 g. The Toothbrush plant extract had a weaker effect, with maximum plant height at 41.00 cm and weight at 16.55 g. Giant milkweed was the least effective extract treatment. Plant height remained low (30.00–33.00 cm), and plant weight was minimal (12.00, 14.00 g). The inoculated untreated control (Control-1), which was exposed to *A. niger* but received no plant extracts, showed the lowest plant height (21.00 cm) and plant weight (10.00 g) throughout the experiment. According to the LSD values ($P < 0.05$) ranging from 1.576 to 1.792 for plant height and 1.554 to 1.751 for plant weight observed differences among treatments were statistically significant. Alphabetical grouping further confirmed the relative efficacy of treatments: Garlic and Neem formed statistically distinct groups from the less effective treatments such as Giant milkweed and Control-1.

a) Control-2, b) Garlic, c) Neem, d) *Aloe vera*, e) Tooth brush plant, f) Giant milk weed, g) Control-1

Fig. 4 Effect of different plant extract on plant growth of safflower

Table 7 Effect of different plant extracts on plant growth of safflower inoculated with *Aspergillus niger*

Treatments	Plant length (cm)			Plant weight (g)		
	D1	D2	D3	D1	D2	D3
Garlic	46.00^{b}	48.00^{b}	50.00^{b}	22.00^{b}	24.00^{b}	25.45^{b}
Neem	42.33^{c}	45.67^{c}	48.47^{b}	19.00^{c}	20.88^{c}	22.00^{c}
Aloe vera	40.00^{d}	42.33^{d}	45.00^{c}	17.00^{d}	18.33^{d}	20.00^{d}
Toothbrush plant	37.00^{e}	39.00^{e}	41.00^{d}	14.00^{e}	15.40^{e}	16.55^{e}
Giant milk weed	30.00^{f}	31.67^{f}	33.00^{e}	12.00^{f}	12.95^{f}	14.00^{f}
Control-1	21.00^{g}	21.00^{g}	21.00^{f}	10.00^{g}	10.00^{g}	10.00^{g}
Control-2	65.00^{a}	65.00^{a}	65.00^{a}	31.00^{a}	31.00^{a}	31.00^{a}
LSD (P < 0.05)	1.792	1.576	1.666	1.751	1.604	1.554

The alphabetical letters show the same homogenous groups in column are not significant with each other.

Impact of several fungicides on safflower plant growth

The impact of pre-sowing fungicidal seed drenches on growth performance of safflower plants inoculated with *A. niger* was evaluated at three different concentrations: 2 g/L (D1), 3 g/L (D2), and 4 g/L (D3). The experiment revealed statistically significant differences (P < 0.05) in both plant length and plant weight among treatments (Table 8). (Fig. 5) Plant length the uninoculated and untreated control (Control-2) showed the highest plant length, maintaining a consistent value of 65.00 cm at all three dosages, serving as a benchmark for optimal plant development. Among fungicide treatments, Dithane M-45 resulted in the greatest increase in plant height under fungal stress, ranging from 53.00 cm at D1 to 59.00 cm at D3. Carbendazim followed closely, with values increasing from 51.50 cm to 56.25 cm, while Topsin-M ranged from 49.33 cm to 53.00 cm. Aliette had a moderate effect (45.00–49.33 cm), and Acrobat was the least effective, with plant heights between 39.00 cm and 43.33 cm. The inoculated, untreated control (Control-1) showed severely restricted growth, consistently recording the lowest plant height (21.00 cm), confirming the negative impact of *A. niger* in the absence of chemical treatment. Plant weight a similar trend was observed for plant biomass. Dithane M-45 led to the highest plant weight among fungicide treatments (26.00–29.00 g), followed by Carbendazim (25.00–27.95 g) and Topsin-M (23.00–26.35 g). Aliette produced intermediate results (19.00–22.88 g), while Acrobat again showed the lowest effectiveness, with plant weights ranging from 17.00 g to 19.50 g. Control-2 remained the best-performing treatment overall (31.00 g), while Control-1 had the lowest weight (10.00 g) across all stages. The Least Significant Difference (LSD) at P < 0.05 ranged from. 1.576 to 1.823 cm for plant height, 1.359 to 1.751 g for plant weight, confirming that all observed differences between treatments were statistically significant. Alphabetical groupings denote homogenous sets within each parameter. Dithane M-45 and Carbendazim consistently formed the top statistically significant group among fungicide treatments, Acrobat and Control-1 consistently formed the lowest performing group.

a) Dithane M-45, b) Carbendazim, c)Topsin-M, d) Aliette, e) Acrobat, f) Control-1, g) Control-2

Fig. 5 Effect of different fungicides on plant growth of safflower.

Table 8 Effect of different fungicides on plant growth of safflower inoculated with *Aspergillus niger*

Fungicide	Plant length (cm)			Plant weight (g)		
	D1	D2	D3	D1	D2	D3
Dithane M-45	53.00^{b}	55.67^{b}	59.00^{b}	26.00^{b}	27.20^{b}	29.00^{b}
Carbendazim	51.50^{b}	53.00^{c}	56.25^{c}	25.00^{b}	25.90^{b}	27.95^{b}
Topsin-M	49.33^{c}	51.67^{c}	53.00^{d}	23.00^{c}	24.50^{c}	26.35^{c}
Aliette	45.00^{d}	47.00^{d}	49.33^{e}	19.00^{d}	21.00^{d}	22.88^{d}
Acrobat	39.00^{e}	42.00^{e}	43.33^{f}	17.00^{e}	18.20^{e}	19.50^{e}
Control-1	21.00^{f}	21.00^{f}	21.00^{g}	10.00^{f}	10.00^{f}	10.00^{f}
Control-2	65.00^{a}	65.00^{a}	65.00^{a}	31.00^{a}	31.00^{a}	31.00^{a}
LSD (P < 0.05)	1.823	1.576	1.701	1.751	1.359	1.496

D1 = 2 g L^{-1}; D2 = 3 g L^{-1}; D3 = 4 g L^{-1}. The alphabetical letters are showing the same homogenous groups in column are not significant with each other

Discussion

Isolation for identification of different seedborne fungi associated with safflower varieties was carried out utilizing the conventional blotter and agar plate techniques. Overall Thori-78 variety showed higher percentage of seedborne incidence whereas; minimum percentage of incidence was seen in S-208 safflower variety. All five fungi were isolated from all four tested varieties excluding S-208 from which *Rhizopus* sp. was not noticed. (Shaker, 2016; Ahmed et al., 2021) isolated twelve different fungi with different frequencies. The findings concerning the germination percentage of both healthy and infected safflower seeds cultivated on moistened blotter paper indicated a decrease in germination for the infected seeds. (Ramesh & Avitha, 2005; Gayathri et al., 2014) also reported that seedborne fungi adversely affect seed germination, crop stand and yield of safflower.

To evaluate the plant extract's in vitro antifungal activity against *A. niger*, fresh, healthy plant material, such as the leaf and bulb of *Aloe vera*, tooth brush plant, garlic, neem and giant milk weed were tested at three concentrations (10, 20 and 30%) against growth of the fungus mycelial. The reaction of the fungus's mycelial development varied greatly with different plant extracts and their respective concentrations. Garlic extract significantly reduced the mycelial growth (14.25-22.50mm) at all three concentrations; the second well effect was noted with neem (17.00-25.50mm). *Aloe vera* and Tooth brush plant were intermediate in their response in order to restrict the growth of the fungus. Minimum antifungal responses (49.00-55.00mm) were seen in usage of giant milk weed. All extracts were more effective at low concentration of solution, as the concentration increased their efficacy was also reduced. Similar response was noticed at mean level of the concentrations of extracts. The findings also showed that, when compared to other treatments, including control, garlic and neem continued to be the most successful. During the study, the impact of plant extracts on safflower growth and development was also evaluated. The findings indicated that there was a considerable variation in plant length between the treatments. In contrast to another control that was infected with the fungal culture but not treated

with any extract of the test plant species, the control treatment, which did not inoculate *A. niger* or apply plant extracts, demonstrated the highest growth of the plants. Significantly highest plant length was observed in pots that were given extract of garlic; the second-best response was seen in neem extract treatment. *Aloe vera* was intermediate in its response followed by tooth brush plant. The response giant milk plant was poor as compared to other treatments of plant extracts. Similar trend was seen in plant weight of safflower for all plant extracts applied in experiment. (Patil et al., 2017) found *Azadirachta indica* and *Ocimum sanctum* effective against seedborne fungi. (Mahmoud et al., 2011) applied extracts from development of neem leaves *Candida albicans, Microsporum gypseum, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger,* and *Aspergillus* terreus *in vitro* condition. Neem extract with 20% ethyl acetate gave significantly highest inhibition percent. (Saniasiaya et al., 2017) found *Aloe vera* with strongest antifungal influence against *Aspergillus niger*.

Five distinct fungicides, namely Aliette, Topsin-M, Dithane M-45, Carbendazim and Acrobat, were evaluated at various concentrations to assess their effects on the fungus. Three concentrations (1000, 1500, and 2000 ppm) of each fungicide were evaluated utilizing the poisoned food technique. The fungus exhibited significant variation in response depending on the type of fungicide used and the concentrations applied. A notable reduction in the radial colony growth of the fungus was observed as the concentration of the fungicide increased. Dithane M-45, Carbendazim, and Topsin M demonstrated optimal effectiveness across all concentrations tested. The elevated levels of Dithane M-45 and Carbendazim effectively inhibited the expansion of fungal growth, demonstrating their efficacy as fungicides, with Topsin M following closely behind. Acrobat demonstrated reduced effectiveness across all three concentrations. The calculated efficacy percentages for these fungicides exhibited consistent trends. Overall, Dithane M-45, Carbendazim, and Topsin M demonstrated the highest effectiveness compared to the other options; however, no significant difference in efficacy was observed at higher doses for these fungicides. The Acrobat demonstrated relatively lower efficacy against the fungus evaluated in laboratory settings. Three applications of various fungicides were administered as a drench prior to the sowing of safflower seeds in pots. All three doses of Dithane M-45 Carbendazim and Topsin M significantly resulted in the highest number of healthy plants, thereby reducing the potential of seedborne fungi in safflower. The findings indicated that there were significant variations in plant length and plant weight across the different treatments. The control treatment, which did not involve the inoculation of A. niger or the application of fungicide, demonstrated the highest plant growth compared to another control that was inoculated with the fungal culture but received no fungicide treatment. In the evaluation of fungicide treatments, the highest plant length and weight were observed with increased dosages of Dithane M-45 and Carbendazim, with Topsin M following closely behind. Aliette showed a moderate response, while the lowest plant length and weight were recorded from pots that received varying doses of Acrobat. (Aggarwal et al., 2005; Sarita et al., 2014; Rauf Bhutta et al., 2001; Nasir, 2003; and Nayak et al., 2018) conducted tests on various fungicides targeting seedborne pathogens, including *Alternaria alternata*, *Curvularia lunata*, *Aspergillus* spp., *Cladosporium cladosporoides*, *Macrophomina phaseolina*, *Drechslera specifera*, *Fusarium oxysporum*, and *Rhizoctonia solani*. Their findings indicated a reduction in pathogen growth and an enhancement in seed germination across multiple crops.

Conclusion and Recommendations

It was concluded from results that the present study effectively demonstrated the presence and impact of seed-borne fungi on different varieties of safflower and highlighted the comparative efficacy of both botanical and chemical control methods. Five distinct fungal pathogens *Aspergillus niger*, *Alternaria carthami*, *Fusarium oxysporum*, *Curvularia* sp., and *Rhizopus* sp. were isolated from four safflower varieties, with *A. niger* emerging as the most prevalent. Among the tested varieties, Thori-78 exhibited the highest incidence of seed-borne fungi and correspondingly recorded the lowest seed germination, plant height, and biomass, whereas S-208 demonstrated the least infection rate and highest germination performance, indicating varietal resistance potential. Botanical extracts exhibited variable antifungal properties. Garlic extract was the most potent among them, significantly suppressing mycelial growth and enhancing seedling vigor across concentrations. Neem extract ranked second, while *Aloe vera* and the Toothbrush plant displayed moderate efficacy. Giant milkweed was the least effective among botanical treatments. Chemical fungicides outperformed botanicals in controlling seed-borne fungi. Dithane M-45 and Carbendazim, particularly at higher concentrations, showed the strongest inhibitory effect on fungal growth and markedly improved plant health parameters. Topsin M followed closely in efficacy, while Aliette provided moderate control. Acrobat was the least effective fungicide in this study. Overall, the findings emphasize the significance of using resistant safflower varieties, such as S-208, and integrating effective seed treatments with potent botanicals like garlic or chemical fungicides like Dithane M-45 and Carbendazim. These integrated strategies can substantially minimize the detrimental effects of seed-borne fungi, ensuring improved germination, growth, and yield potential in safflower cultivation. The results suggest that less frequency of seedborne fungi was seen in S-208 variety as compared to others hence recommended for cultivation. Garlic and neem were found better in control of predominant fungus isolated from safflower seeds hence suggested as seed treatment and soil drench to minimize the losses occurred by seedborne pathogens. Dithane M-45, Carbendazim and Topsin M were best at their all concentrations hence suggested for management of seedborne fungi of safflower.

Conflict of Interest: The authors declare that they have no conflict of interest.

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