Research Article

Exploring the Potential of Castor Oil for Effective Management of Seed-Borne Fungi in *Zea mays*

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Abstract | An experiment was conducted in a Plant pathology laboratory at Women University of Swabi in Khyber Pakhtunkhwa, Pakistan, to explore the management of seed-borne fungi in maize using castor oil treatment. The study involved collecting and analyzing seeds of various maize varieties such as yellow, blue, white, waxy, and hybrid, sourced from Buner district in KP. Through blotter test and agar plate methods, a total of eight different species of fungi were isolated from five different maize varieties. In the blotter test method, only spores and mycelium of seed-borne fungi were detected, that was further confirmed by the agar plate method revealed the presence of eight different types of fungal colonies in maize varieties, each with varying percentages. Like *Trichoderma harzianum* colonies accounted for 3.33% of the treated seeds and 13.33% of the untreated seeds. Similarly, *Penicillium* spp. infection% 13.32% of untreated seeds and *Aspergillus niger* accounted for 13.32% significantly was less compared to untreated seed. It appears that *Fusarium semitectum* was found in 13.33% of untreated seeds, while *Alternaria solani* accounted for 6.66%. However, in the treated varieties, both of these fungi did not appear. *Aspergillus niger*, on the other hand, had the highest colonies in both treated (46.64%) and untreated (56.66%) seeds. Additionally, *Fusarium oxysporum* was present in 16.66% of treated seeds and 49.98% of untreated seeds, while *Fusarium moniliforme* was found in 6.66% of treated seeds and 3.33% of untreated seeds. In this experiment, when comparing the germination percentages of the treated (with castor oil) and untreated seeds across all varieties, the LSD (least significant difference) analysis showed interesting results. By targeting and treating the maize seeds with castor oil, it could potentially help mitigate the adverse effects of these fungi and contribute to a healthier and more productive crop. This study aimed to investigate the effectiveness of castor oil in managing seed-borne diseases caused by these fungi. This is a promising direction for future research and application in agricultural practices to ensure the seed safety.

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Introduction

Maize (*Zea mays*) is also called corn in some states (Oxford English Dictionary, 2012). It is very essential yearly crop found all over the world and belong to family Poaceae. *Zea mays* word derived from two different languages zea from Greek which means supporting or encouraging life and mays from Taino language which general means life provider or benefactor. Other names also used for maize like silk maize, makka, barajovar, etc. (Kumar and Jhariya, 2013). After rice and wheat, maize is the chief crops of all over the world which used many states as a fundamental food (Sandhu *et al*., 2007). According to Fernandez and Felipe (2011) maize is more water proficient crops other than soybean etc. and implanting mostly in summer season.

The international fabrication of maize according to the survey of 2014 is 1,04 billion tonnes. Maize is used both for feedstuff and it is 3rd most important cereal crop (Ali *et al*., 2020a, b; Adnan and Bilal, 2020). In United States of America (USA) the maize fabrication is very high due to their highest production it is called the internationally sovereign or queen of grains (Torban and Alli, 2021).

For any upright yields fabrication, the handiness of good class seeds of great springy selections, infection permitted approved to the developing zone, and predilection by the agriculturalist, is required because moulds and other sorts of pathogens that outbreaks cultured crops and cause a various diseases due losses their production and also reason a very serious financial effect on agronomy fabrication (Saqib *et al*. 2016; Thieken *et al*., 2016; Zulfiqar *et al*., 2016). Seed-born disease also a type of greatest vital specific plants bug that is transferred through seed meanwhile for virtuous yield and plant inhabitants seed vigour is the utmost significant aspect in recent discipline (Rahman *et al*., 2008). Seed born pathogen is one of the most totalling complications to answerable for the re-entrance of infection of the previous as well as the primer of infection keen on novel region (Walcott *et al*., 2003). According to a couple of botanist (Van *et al*., 2001; Rajput *et al*., 2005; Niaz and Dawar, 2009) seed born pathogen are universal evils. They are liable for the demise of grains in both post-development and growth stages and too deviation in plant morphology. In Maize crops many diseases caused by various seed born fungi spp. like *Alternaria alternata*, *Aspergillus* spp., *Bipolaris maydis*, *Fusarium moniliforme*, *Fusarium* spp., *Cephalosporium* spp. *Helminthosporium* spp., *Mucor* sp., and *Penicillium* species as a result cause losses of maize production (Anne *et al*., 2000; Dasjardin *et al*., 2006). There are numerous abiotic and biotic factors that limit the harvest in maize and result in many diseases as well as poor crop management. Maize plants are unnatural by a large number of infections the most vital of which are fungal and bacterial pathogens that are present in various fields of Pakistan. Maize is disposed to several fungal diseases, to a rarer group of bacteria, viruses, many other mycoplasma-like organisms, nematodes, and various other higher parasitic plants. The various bacterial and fungal species have been reported as disturbing for maize crops (Cobo-Díaz *et al*., 2019).

An experiment was conducted by Chuken and Enyinkwn (2016) to determine the seed born fungi in maize seed (yellow variety) and isolated the fungi by used of potato dextrose agar (PDA) method and observed *Aspergillus falvus*, *Fusarium oxysporium*, *Rhizoctonia stolonifer*, *C. lunata*, and *B. theobromae*. One of these fungus *the F. flavus* is frequently occurring fungi in this study. They also showed that these fungi began the germination disaster 5% in tested seed and also effected seedling exclusion of *R. stolonifera* as a consequential instigated sign on the plants.

Another experiment was conducted by Hussain *et al*. (2012), to isolate the pathogenicity of fungi species from different localities of maize seeds (Azad Jammu and Kashmir (AJK) in which also the most frequently appeared fungi species *Fausarium* (87.25%) and *Aspergillus* (82.50%) were detected individually. *Fusarium moniliforme* began various infections in maize seeds like ear deterioration, germ rot, shoot decay, plantlet stain, seed decay, droop and stunt. Comparable *Aspergillus niger* also caused ear rot infection (Hussain *et al*., 2012). The goals of this study was to scan the effect of castor oil on the germination of maize (*Zea mays*) seeds and also reduced the infection of seed born fungi.

Many oils, like Thyme, Oregano, Pennyroyal, cumin essential, Peppermint oil etc also been shown to be effective against fungi (Pinto *et al*., 2006). Pennyroyal oil (*Mentha pulegium*) antifungal activity has also been proven in numerous publications. A MIC at 90% inhibition was demonstrated against *Candida albicans* between 6.2 and 7.5 µL/mL (Schmidt *et al*., 2007).

Well known for its use as a spice, cumin oil (*Cuminum cyminum*) At present, it is also being considered for its antifungal activity. The study by Kedia *et al.* (2014) on fungal insolates from food samples, containing various strains of *Aspergillus* spp. and *Fusarium* spp. in particular, demonstrated an activity against these strains with a concentration of 0.6 µL/mL of cumin essential oil (Kedia *et al.*, 2014). Peppermint essential oil (*Mentha piperita*) is already well known for its antifungal activity has often been proven, for example, a MIC of 500 ppm completely inhibits the growth of *Candida albicans* (Tampieri *et al.*, 2005).

Materials and Methods

Collections of seed samples

For this trial, a total of five different maize varieties, V1(Waxy corn), V2(White), V3(Bllue), V4(Hybrid), V5(Yellow) were collected from district Buner. Two local varieties, namely white and hybrid maize, were collected from Sura village in the same district. Additionally, three varieties, including Blue, Yellow, and Waxy corn, were obtained from an agriculture shop (as shown in Figure 1). These specific seeds were chosen due to their ease of propagation and convenient handling during experimental work.

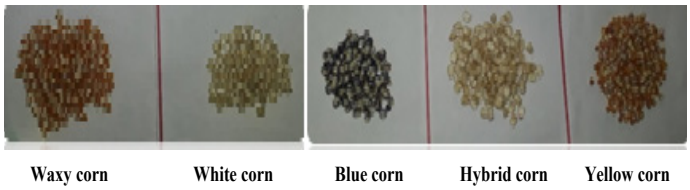

Figure 1: *Five different varieties of maize seeds.* $LSD_{0.05}$ *= 34.64*

Treatment of seeds

To ensure the elimination of surface impurities, all maize varieties underwent surface sterilization using a 70% ethanol for one minute. Following this, glass petri dishes were thoroughly washed with water and subsequently sterilized. The sterilization process involved placing two layers of blotting paper and one layer of cotton on the petri dishes, which were then subjected to a temperature of at least 120 °C for duration of one hour.

A random selection of 60 seeds was made from each maize variety. Out of these 60 seeds, 30 were treated with castor oil, while the remaining 30 were left untreated. This was done to examine the impact of castor oil on the germination of maize seeds.

Germination test by blotter method

According to the methodology outlined by the International Seed Testing Association (ISTA, 1996), the blotter method was employed. Three layers of blotting papers were cut to fit the size of the glass Petri dishes. These papers were then soaked in sterile water and positioned at the bottom of each petri dish. For each variety, thirty treated and thirty untreated seeds were selected and placed uniformly on the moist blotting paper, with a rate of 10 seeds per dish. In total, thirty plates were prepared, consisting of three treated and three untreated plates for each variety. You can refer to Figure 2A and B for a visual representation of the plates and incubated at 20-24 degree celsius in irregular cycle of 12/12 hours' light and darkness per 7 days and recorded the germination% of infected and unaffected seeds.

Isolation and identifications of fungi by blotter test method

After incubating for seven days, the samples were examined under an electron microscope. By using identification keys and cross-referencing with each seed plate, it was observed that all varieties, both treated and untreated with castor oil, displayed the presence of various types of fungi on spoiled seeds in all three plates. Furthermore, it was noticed that certain important seed-borne fungi were completely absent on these seeds. To investigate the absence of these fungi, the researcher employed the potato dextrose agar method on all 30 plates containing spoiled seeds. This method was used to identify fungal growth on different varieties of maize. Additionally, the germination percentage of the maize seeds was recorded for further analysis.

Isolation and identifications of fungi by PDA plate method

Researcher used agar plate method for the reason that some important seeds born fungi not appears on blotter paper. Therefore, in agar plate method the entire spoiled seeds (infected seeds) were plated on the petri dishes containing potato dextrose agar medium (5%) then incubated for 6 to 7 days at 20-25 °C. After 7 days of the incubation period the fungi were examined under electron microscope and identified. Based on colonies characteristics and morphological structures of the fungus spores all types of fungus examined under compounds microscope Mathur and Kongsdal (2003).

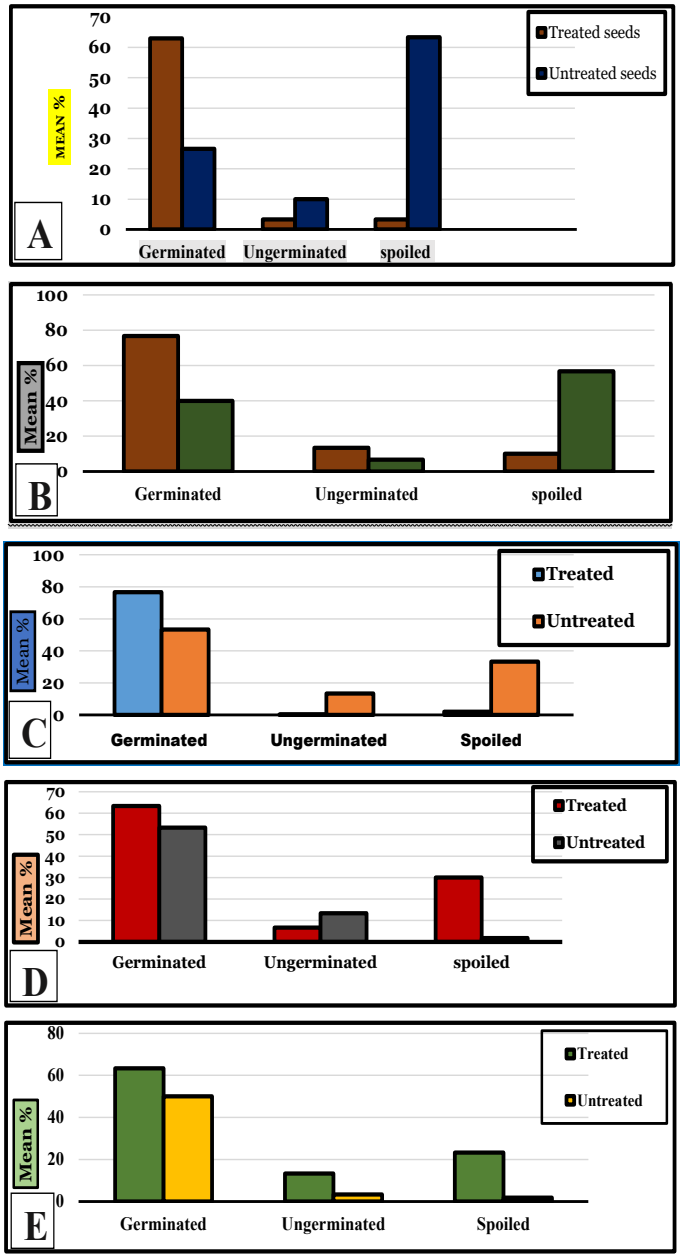

Figure 2: *(A) Comparison of seeds germination b/w treated (castor oil) and untreated variety of yellow corn. $LSD_{0.05}$ = 37.56. (B) Comparison of seeds germination b/w treated (castor oil) and untreated variety of white corn $LSD_{0.05}$ = 3.84. (C) Comparison of seeds germination b/w treated (castor oil) and untreated variety of blue corn $LSD_{0.05}$ = 28.48. (D) Comparison of seeds germination between treated (castor oil) and untreated variety of waxy corn $LSD_{0.05}$ =26.4. (E) Comparison of seeds germination between treated (castor oil) and untreated variety of Hybrid corn.*

Statistical analysis

Data were analyzed by using one way analysis of variance (ANOVA) which was followed by the least significant difference (LSD) at $P_{(0.05)}$. All analysis was performed using IBM SPSS STATISTICS program (Sokal and Rohlf, 1995).

Rsults and Discussion

Germination percentage of maize seeds

The germination percentages of maize treated with castor oil and untreated seeds are varied expressively from sample to sample. Figure 2A, B, C, D, E and 3 shows that the highest germination percentage when treated with castor oil while the untreated seeds showed lowest germination percentages. The same highest germination % on treated seeds were recorded in white and blue corn (76.66%) (V2, V3) (Figure 2B, C) and Figure 3 the lowest % were recorded in waxy corn (3.33%) (Figure 3d) and moderate same percentage showed in yellow and hybrid corn (63.33%) (Figure 2A, E) and Figure 3.

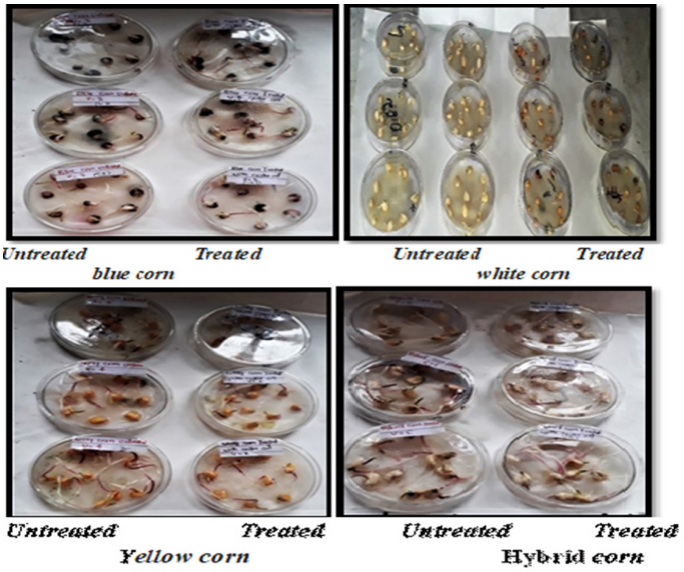

Figure 3: *Visual representaion of maize varities Germination on blotter test method.*

While is the germination percentages of untreated seeds of yellow (26.66%), white (40%), blue (53.33%) and hybrid corn (50%) is lesser than castor oil treated seeds. But in case of waxy corn the untreated seeds percentages are 66.66% and treated seeds percentage is 63.33% (Figure 2A, B, C, D, E).

The germination parentage of all varieties were measured and recorded, yellow corn germination% of treated seeds were 63.33% and untreated 26.66%, V2 White corn germination percentage of treated 76.66% and untreated 40%, V3 Blue corn germination percentages of treated 76.66% and untreated 53.33%, V4 waxy corn germination percentages of treated 63.33% and untreated 66.66%, and V5 Hybrid corn germination percentage of treated 63.33% and untreated 50% so these above five varieties the V2

and V3 showed maximum germination % Figure 2A, B, C, D, E and 3.

Effect of castor oil on Seed borne fungi by blotter test method

In blotter test method the researchers were recorded the highest infection percentages in maize untreated seeds is compare to the castor oil treated seeds. Highest infection percentage were recorded in yellow corn (63.33%) in untreated seeds while their treated infection % is 33.33% and the lowest infection percentages were recorded in treated white corn (10%) and their untreated % is 56.66%. Blue corn (20%), waxy corn (30%) and hybrid corn is 23.33% infected in case of castor oil treated seeds. As compare to treated castor oil infection percentages the untreated seeds of blue corn are 33.33%, waxy corn 20% and the hydride corn is 46.66% infected. Waxy corn is definite case in which high rate of infection percentage take place in treated castor oils as compare to untreated seeds.

Effect of castor oil on seed borne fungi by PDA plate method

In this method 8 different types of seed born fungi; *Trichoderma harzanium, Fusarium oxysporum, Alternaria solani, Aspergillus niger, Peculium* spp., *aspergillus Flavus, Fusarium semitectum, Fusarium moniliforme* were isolated from five maize local varieties which collected from district Buner. *Fusarium oxysporum* are reported 33.32% in treated seeds and 49.98% in untreated seeds (Figure 4A, B). In 49.98% the colonization percentage of *Fusarium oxysporum* in yellow corn 3.33% and white and hybrid corn 6.66% infected but waxy and blue corn not affected by this spp. (Figure 4A, B). Is compare to treated seeds varieties the untreated seeds all sample is infected by this particular fungus like yellow corn 16.66%, hybrid and white corn 10%, waxy (sweet corn) and blue corn 6.66% (Figure 4A, B). highest percentage of *penicillium* spp. also were recorded in untreated seeds (32.32% all sample %), in 32.32% the same colonization percentage of yellow, white and blue corn 3.33% and waxy and hybrid corn 6.66%. while in castor oil treated seeds verities the *penicillium* spp. not detected in all varieties. in blue and hybrid corn 3.33% and waxy corn 6.66% *Penicillium* spp. appears and completely absent in yellow and white corn so the over-all colonization percentage of treated seeds is 13.32% (Figure 4A, B). In this current studies the two types of seed born fungi also identify in very small percentages one is *Fusarium semitectum* which detected just untreated waxy corn (13.33%) and white corn 10% and completely absent other treated and untreated samples .and the second type is *Fusarium moniliforme* which also detected only 2 varieties one is blue corn (6.66%) treated and the other is waxy corn untreated (3.33%) (Figure 4A, B).

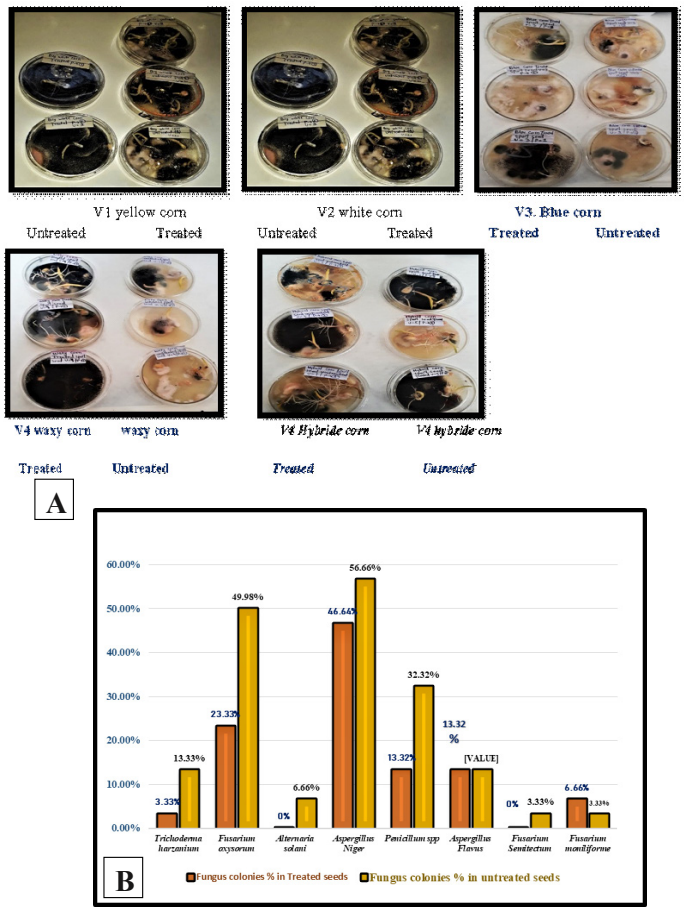

Figure 4: *(A) visual representation of Pathogenic stage of maize varieties in agar plate method. (B) Colonization% of seed born fungi when treated with castor oil.*

Seed-borne pathogens refer to microorganisms such as fungi, bacteria, and viruses that reside on or within seeds. These pathogens can have various effects on plants and crop production. The effects of seed-borne pathogens can be detrimental. They can lead to reduced seed germination, resulting in poor plant establishment. Seedling emergence may also be affected, leading to stunted growth or even death of young plants. This can result in significant economic losses for farmers. It is important to implement measures such as proper seed treatment, seed certification programs, and crop rotation to minimize the risks associated with seed-borne pathogens and ensures healthy and productive crops.

The current study was to find out the pathogens associated with seeds. The study of agar plate method was designed to resolute the most important seed born fungi which not appears in blotter test method. Followed Khan *et al*. (1988) the agar plat method is frequently appropriate for the detection of seed born fungi. According to Orisi *et al*. (2000) and Ghiasian *et al*. (2004) in Pakistan and other tropical area the *Penicillium*, *Fusarium*, and *Aspergillus niger* is found frequently. In this present studies *Penicillium, fusarium* and *Aspergillus* spp. also appeared in maize treated and untreated seeds samples in agar plate method.

Aspergillus flavus fungi was isolated in agar medium this information reported by Sitara and Akhter (2007). While similar results were obtained in our current study that *Aspergillus flavus* colonies also isolated from all tested varieties of seeds. *Fusarium moniliforme* was recorded the most effected pathogens of maize seeds (Caldwell *et al*., 1981). *Fusarium moniliforme* also supplementary declining pathogen as compare to *Aspergillus niger* by this pathogen caused many diseases like damaged of roots emerging germ lines, necrosis of adjacent roots of seedling and rotting sickness of seeds. Seeling production was also effected by *Fusarium moniliforme, Aspergillus* spp. and *Penicillium* spp. (Ibrahim and Farg, 1965). *Aspergillus niger* most recurrently isolated fungi which affect the seeds (Arif and Ahmad, 1969).

In present study *Aspergillus niger* was isolated from all treated and untreated varieties of *Zea mays* Conferring to Mathur et al. (1975) the *Trichoderma harzanium* also new reported maize seed born fungi which debauched grow in agar medium as compare to the blotter test method. They isolated this *Trichoderma harzanium* from sorghum seeds in agar plate method. In this contemporaneous studies *Trichoderma harzanium* also were recorded very lowest rate and their colonization percentage is 3.33% in untreated seeds of yellow corn and 13.33% colonies appears in untreated yellow corn. The *Trichoderma harzanium* is more frequently detected from untreated yellow corn as compare to treated seeds. *Alternaria solani* also the most important seeds born fungi and also reported in untreated yellow corn.

Apart from that in blotter test some fungal flora *Aspergillus flavus*, *Aspergillus niger, Fusarium* species and *Alternaria solani* were detected based on colony morphology and these were further identified under the compound microscope based on spore/conidial morphological studies varieties. Similar results were obtained by Mathur and Kongsdal (2003), and Agarwal *et al*. (1990) Likewise, Sreenu *et al*. (2019) also identified Five fungal flora *Aspergillus flavus, Aspergillus niger, Fusarium moniliformae, Curvularia lunata* and *Alternaria alternata* using blotter test method.

Conclusions and Recommendations

Eight different types of seed born fungi appeared in five types of Zea maize verities. In agar plate method the highest no of incidence of *Aspergillus niger* (56.66%) was recorded in untreated and 46.65% treated seeds. While *F. semitectum* and *Alternaria solani* completely absent in treated seeds of maize. This report also indicated that the harmful fungi of plant can be controlled by castor oil treatments. Oil treatment method is very effective and need to be further research in the field of medicine so that farmar can controlled the seedborn fungi.n

Acknowledgement

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Novelty Statement

In this research study, we explored the use of castor oil as a potent and eco-friendly solution for effectively managing seed-borne fungi in Zea mays. By binding the unique properties of castor oil, we unlock a novel approach towards protection of maize crops, offering a sustainable and effective alternative to existing methods. Our research provided the way for enhanced seed health and improved crop productivity.

Author's Contribution

Rahmat Elahi: Performed research work.
Gulnaz Parveen: Supervised and conceived the idea.
Nazara: Help in writing paper.
Faryal Ali: Data entry in SPSS.
Nain Tara and Saba Iqba: Helped in research work.

Data availability
All data has been provided no hidden data.

Ethical approval
Not applicable to this paper.

Conflicts of interest
The authors have declared no conflict of interest.

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