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Comparative Efficacy of Physico-Chemical and Biological Treatments on Seed Germination and Mycoflora of *Sesamum indicum* L.

[1]Brian Gagosh Nayyar, [1]Abida Akram, [1]Shaista Akhund, [1]Wajiha Seerat and [2]Sehrish Sadia

[1]Department of Botany, PMAS-Arid Agriculture University, Rawalpindi, 46300, Pakistan
[2]College of Life Sciences, Beijing Normal University, Beijing 10087, China

Abstract: *Sesamum indicum* L. is among the oldest oilseed crops of the world. Its production and storage are affected by a vast group of microbes, primarily the fungi. The present investigations were conducted with the aim to address agronomic performance traits of seed quality. Many deteriorative microbes, particularly fungi, create problems in its production and storage. So, the present study was designed to evaluate the effect of different treatments to increase the germination and inhibit the mycoflora of sesame seeds. Sesame seeds were subjected to various treatments, including, thermotherapy (by incubation at 50ºC, 60ºC and 70ºC), application of fungicides (Mancozeb, Thiophante Methyl and Carbendazim), plant extracts (*Melia azedarach*, *Cannabis sativa* and *Pongamia pinnata*) and bioagents (*Trichoderma viride, T. harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Saccharomyces cerevisiae*). Seed germination was tested by following the protocol of International Seed Testing Association. Antifungal activity was tested by poisoned food technique. The results revealed that the germination increased effectively, due to thiophanate methyl up to 46%, followed by *Cannabis sativa* (37%). Thermotherapy increased germination (28%) at 60ºC but caused harmful effect on seeds at 70ºC, whereas, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma harzianum* increased germination up to 40%. Out of 7 fungi tested against fungicides and plant extracts, 4 fungi, namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium egyptiacum*, were inhibited up to 100% by Carbendazim and Thiophanate Methyl, while Mancozeb inhibited *Penicillium egyptiacum* (100%), only. *Cannabis sativa* inhibited *Alternaria alternata* (100%) and *Rhizopus oryzae* (55.6%). Overall treatment with fungicides gave best results but they were not eco-friendly. So, this study recommends the use of plant extracts and bioagents, which may increase seed germination and inhibit seed-borne fungi without any harmful effect.

Key words: Biological control, Fungicides, Thermotherapy, Sesame.

INTRODUCTION

Sesame holds a special importance in the world's oil production, due to its high quality. Sesame seeds comprise 40-60% of the oil. It is highly rewarding crop due to its low cost of production and high market price (Anwar *et al.*, 2012). In spite of the multidimensional uses of sesame, its cultivation in Pakistan is very discouraging with very low seed yield per hectare. The demand for edible oil is increasing with an increase in population, but the production of edible oil is decreasing every year (Bhatti *et al.*, 2005). The local production estimated at 0.680 million tonnes, meets only 24% of the domestic requirement of edible oil, while the remaining 76% is met through imports. Total availability from all sources is provisionally estimated at 1.749 million tonnes (GOP, 2010). Low yield of sesame, in Pakistan, may be attributed to an attack by various pathogens, among them fungi play a dominant role in decreasing quality and longevity of the seeds. Seeds play a vital role in transporting the pathogens, which are associated internally or externally with the seed. Seed-transmitted pathogens cause diseases at different phases of crop growth from germinating seed up to crop maturity and heavy losses, caused by seed-borne pathogens in various crops have been observed. Storage fungi slowly kill the embryos of the seed they invade, seedlings raised from such seeds lack the normal vigour. The effects of fungal attack on seeds include a reduction in potential of germination, development of visible moldiness, discoloration, bad odour, loss of dry matter, heating, chemical and nutritional changes, loss of quality and the production of mycotoxins (Braghini *et al.*, 2009), which are hazardous to humans and livestock.

Antimicrobial efficiency of plant extracts (Sen and Batra, 2012; Lone and Lone, 2012; Johnson *et al.*,

***Corresponding Author:** Brian Gagosh Nayyar, Department of Botany, PMAS-Arid Agriculture University, Rawalpindi, 46300, Pakistan
brian_gagosh@hotmail.com

2011), fungicides (Palakshappa *et al.,* 2012; Rajput *et al.,* 2006; Gondal *et al.,* 2012) and different antagonists (Anbuselvi *et al.,* 2010; Csutak *et al.,* 2013) have been reported against many fungal pathogens by several workers all over the world. Among them, biological treatments give reasonable and comparatively eco-friendly management strategy as compared to other strategies. Keeping in view, the present study was undertaken to compare different strategies and find out the appropriate treatment and its doses to see its impact on the germination of seeds as well as to eliminate seed-borne mycoflora of sesame. Higher and healthy production of sesame may contribute towards edible oil industry and meet country's requirement which may help in reducing its import.

MATERIALS AND METHODS

Plant Material: Fifteen seed samples of sesame were obtained from retail markets of Sialkot, Pakistan. All seed samples were tested for mycoflora count (Nayyar *et al.,* 2013), seed viability and pathogenicity test (Nayyar *et al.,* 2014). In this study, the most contaminated sample was subjected to physical, chemical and biological treatments to check its efficacy on seed germination and mycoflora control.

Seed Germination:

Thermotherapy: Sesame seeds were subjected to various temperatures, i.e., 50, 60 and 70 ºC, for two hours in a dry heat oven. 75 seeds were taken from each treatment while the seeds without any heat treatment served as the control (Gama *et al.,* 2014).

Seed dressing fungicides: Chemical fungicides, namely, Mancozeb, Thiophante methyl and Carbendazim, were purchased from authorized dealer of the local market of Rawalpindi. Seeds were dressed separately at 0.3, 0.2 and 0.1 g/kg concentration, whereas untreated seeds served as control.

Methanolic plant extracts: Fresh leaves of *Melia azedarach* (L.), *Cannabis sativa* (L.) and *Pongamia pinnata* (L.) were collected from PMAS-Arid Agriculture University, Rawalpindi, and immediately brought into the laboratory, where they were washed thoroughly with tap water followed by distilled water and air-dried at room temperature**.** After drying, the leaves were ground to fine powder and 10 g powder of each plant was mixed with methanol to make 100 ml volume of methanolic extract. Then these extracts were placed on a shaker for twelve hours, filtered through muslin cloth and concentrated in a water bath. Tenfold dilutions (10, 1 and 0.1%) were prepared and seeds were immersed in each concentration for twenty minutes, whereas seeds immersed in distilled water served as control.

Biocontrol agents: Fungal (*Trichoderma viride* Accession # FCBP 232 and *Trichoderma harzianum* Accession # FCBP 249), and bacterial cultures (*Pseudomonas fluorescens* Accession # FCBP 188 and *Bacillus subtilis* Accession # FCBP 189) were obtained from the First Fungal Culture Bank of Pakistan (FCBP), University of the Punjab, Lahore, Pakistan, while yeast (*Saccharomyces cerevisiae* Accession # NCYC 505), used in this experiment, was obtained from National Collection of Yeast Culture, UK. The cultures obtained from culture banks were reactivated. The bacterial strains were grown for 48 hours in Luria Broth **(**LB**)** with constant shaking. The yeast was also grown under the same conditions in a medium consisting of yeast extract, 4 g; nutrient broth, 8 g; glucose, 1.5 g per litre. Fungal strains were cultured for 10 days on Potato Dextrose Agar. To sporulating cultures 5-10 ml of sterile distilled water was added, the surface of the cultures was scraped with a spatula and the resulting suspension was transferred into a beaker. Seeds were dipped in each microbial suspension for 20 minutes. The seeds dipped in distilled water served as control (Schmitt *et al.,* 2009).

Twenty five seeds from all treatments were sown in plastic pots of 10 cm diameter and incubated at 25-28 ºC for 2 weeks. The inhibition against seed-borne mycoflora was examined and the germinated seeds were counted. The percentage of germination was calculated by using the following formula:

$$\text{Germination (\%)} = \frac{\text{No.of seeds germinated}}{\text{Total no.of seeds}} \times 100$$

Inhibition of seed mycoflora:

Test species: Seven test species viz. *Alternaria alternata, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Cercospora* sp., *Penicillium egyptiacum* and *Rhizopus oryzae* were selected from the mycoflora isolated in the previous study from sesame seeds, on the basis of their prevalence (Nayyar *et al.,* 2013).

Poisoned food technique: Poisoned food technique (Dhingra and Sinclair, 1993) was used to evaluate *in vitro* inhibitory effect of fungicides, bioagents and plant extracts against test species. Each concentration was dissolved in PDA (20 ml). After the medium solidification in autoclaved Petri dishes, small inoculums were taken from 5 days old culture of each test species and placed in the center of Petri dish. The antifungal drug "Terbinafine" was used as positive control, while methanol was used as negative control. All Petri dishes were incubated at 28±2ºC and the radial growth of the colony was measured after 7 days of incubation. The percentage inhibition was calculated using the following formula and the percent inhibition of mycelial growth was determined.

$$\text{Inhibition (\%)} = \frac{\text{growth in control} - \text{growth in treatment}}{\text{growth in control}} \times 100$$

Statistical analysis: All the data were subjected to Analysis of Variance (ANOVA) and Tukey's HSD by using SPSS, 16.0 for windows (SPSS, Chicago, IL-USA).

RESULTS AND DISCUSSION

Seed germination:

Thermotherapy: Naturally infected seeds of sesame were evaluated in order to study the effect of different temperatures on seed germination (Fig. 1). It was observed that the treated seeds at 50 °C gave 13% increase in germination as compared to control, whereas those at 60 °C gave 28% increase over control, while at 70 °C the rate of germination decreased.

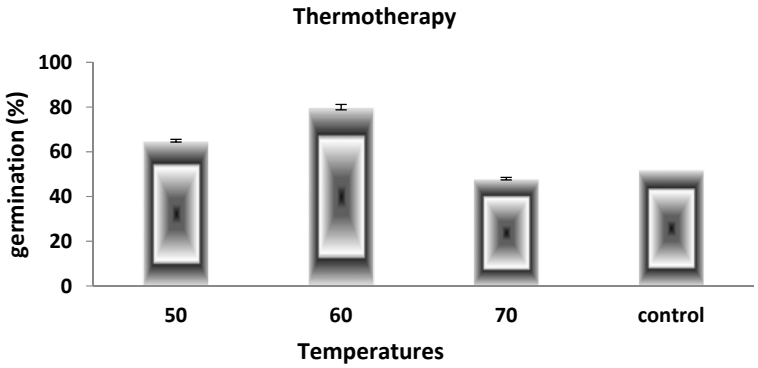
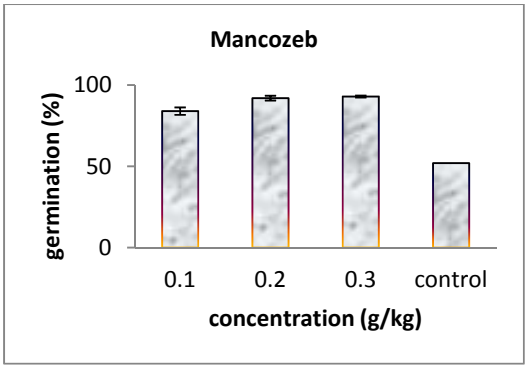


Fig. 1: Effect of various temperatures (ºC) on seed germination.

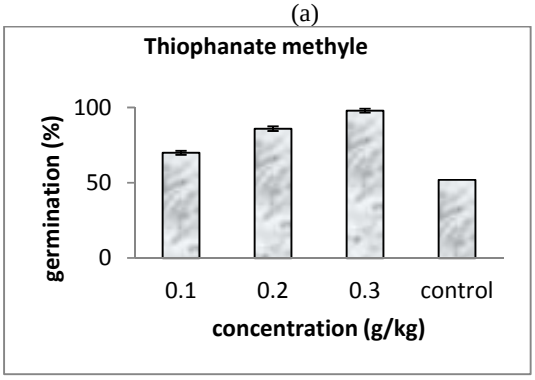
Seed dressing fungicides: The seed germination increased due to the treatment of fungicides as compared to control. It was 84-93% with treatment of Mancozeb, 70-98% with Thiophanate Methyl and 73-93% by treating with Carbendazim (Fig. 2). Thiophanate methyl was found to be the most effective, which increased the germination rate by 18-46% and showed a significant increase among three doses in the same treatment. Mancozeb showed 32-41% increase in all three doses, while Carbendazim had increased germination by 21-40%; however, it showed no significant increase among doses.

Plant extracts: Three extracts of *Melia azedarach*, *Cannabis sativa*, and *Pongamia pinnata* were tested on germination of sesame seeds. *C. sativa* was found to be the most effective and showed a significant increase in seed germination as compared to the control, i.e., seeds without treatment of plant extracts. 89% germination was observed in seeds treated with 10% dilution of *C. sativa*, exhibiting 37% increase in seed germination. Further dilutions of 1 and 0.1% showed germination up to 77 and 72%, respectively (Fig. 3). *M. azadirachta* increased germination up to 32 percent, while *P. pinnata* was found to be less effective as it exhibited 12% increase in germination.

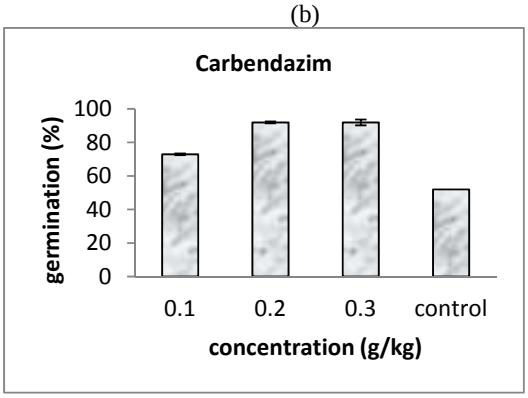
Biocontrol agents: The treatment of bioagents increased the rate of seed germination and healthy seedlings as compared to control. The most promising effect on germination, i.e., 92% was shown by *P. fluorescence*, *B. subtilis*, and *T. harzianum* by increasing germination up to 40%. Although, yeast treatment increased germination by 30% but it provided healthy seedlings up to 78%, which is the highest rate, not given by any other bioagents (Fig. 4). *P. florescence* showed 64.4% healthy seedling, while *T. harzianum* did not increase seedling health rather it increased the rate of germination.



(a)



(b)



(c)

Fig. 2: Effect of various concentrations (g/kg) of fungicides (a-c) on seed germination.

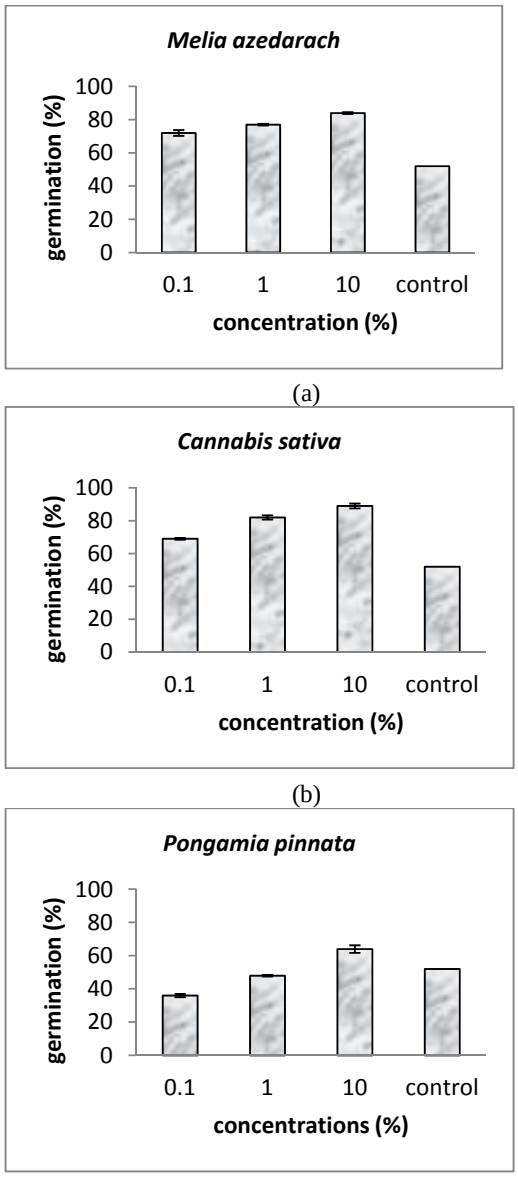


Fig. 3: Effect of various concentrations (%) of plant extracts (a–c) on seed germination.

Inhibition of seed mycoflora:

Fungicides: The data on mycelial inhibition is shown in Fig. 5. Among all treatments, Carbendazim was found to be the most effective fungicide, which showed 100% inhibition of four fungi, namely *A. flavus*, *A. niger*, *F. oxysporum* and *P. egyptiacum* at all concentrations. Thiophanate methyl was also effective by showing 100% inhibition of three fungi viz. *A. flavus*, *A. niger* and *P. egyptiacum*, at all concentrations, however, *F. oxysporum* was 100% inhibited only at a dose of 0.3 g/kg. Mancozeb was less effective fungicide that showed maximum inhibition against *A. niger* (91%) and *P. egyptiacum* (90.6%) at the highest dose. All the treatments showed highly significant results and reduced mycelial growth except *R. oryzae*, which was not inhibited by any fungicide.

Plant extracts: Quite significant results were obtained from all treatments of plant extracts (Fig. 6). Among three plants studied, *C. sativa* was found to be the most effective against fungi in poisoned food technique. It reduced the mycelial growth of *A. alternata* up to 96.88 %, followed by *Cercospora* sp. (74.57%) at 10% dilution. Even this extract inhibited *R. oryzae* up to 46.17% at 10% dilution, which had not been inhibited by any other extract and fungicide. *M. azadirachta* showed moderate antifungal activity, it highly inhibited *A. alternata* (93.2%), followed by *Cercospora* sp. (67.15%) and weakly inhibited the rest of the fungi at 10% dilution. However, *P. pinnata* showed low antifungal activity as compared to other extracts.

Heat treatment of seeds is one of the potential approaches to increase seed germination and control certain plant diseases. In the present study, it was observed that the rate of germination was the highest at 60 ºC and the lowest at 70 ºC, hence, it was concluded that an increase in temperature caused harmful effect on the viability of seeds (Cancino *et al.,* 1993). Therefore, 60 ºC was considered as the best temperature treatment for sesame seeds. Bennett and Colyer (2010) reported that thermotherapy is the most effective treatment for removing *Fusaria* from cotton seed with minimal loss of germination and seed vigour and it may be optimized to prevent fungal infection. The seeds treated with fungicides showed better results, especially in the case of Thiophanate methyl, which was found to be the best in increasing the germination rate. The results are dissimilar with the findings of Tomar *et al.* (2012), who found Carbendazim as an effective fungicide. A similar case was also reported by Dhanamanjuri *et al.* (2013), they reported carbendazim as the best treatment for the seed germination of *Cicer arietinum* and *Zea mays*. However, seed treatment with different biocides (bioagents and plant leaf extracts) has been reported to be the safest in comparison to fungicides (Gupta *et al.,* 2012). *C. sativa* was found to be the most effective plant for an increase in germination, while *P. pinnata* was less effective. However, Mamun and Shahjahan (2011) reported that *P. pinnata* showed a significant increase in wheat germination with an average percentage of 95.27. The effect of *M. azadirachta* and *C. sativa* on seed germination has not been reported so far. Biological control had attained importance in modern agriculture to curtail the hazards of the intensive use of chemicals for disease control. The bioagents stimulate the plant growth, the induction of systemic resistance of plants to pathogens, phytohormones production and improvement of nutrients and water uptake. Several biocides have been reported to increase seed germination and vigour index (Bharath *et al.,* 2005). All the bioagents tested in this study were found effective, as shown in previous data. In support of present findings, Bharath *et al.* (2005) reported that the seed treatment with *T. harzianum* and *T. viride* improved the seed germination, seedling vigour and

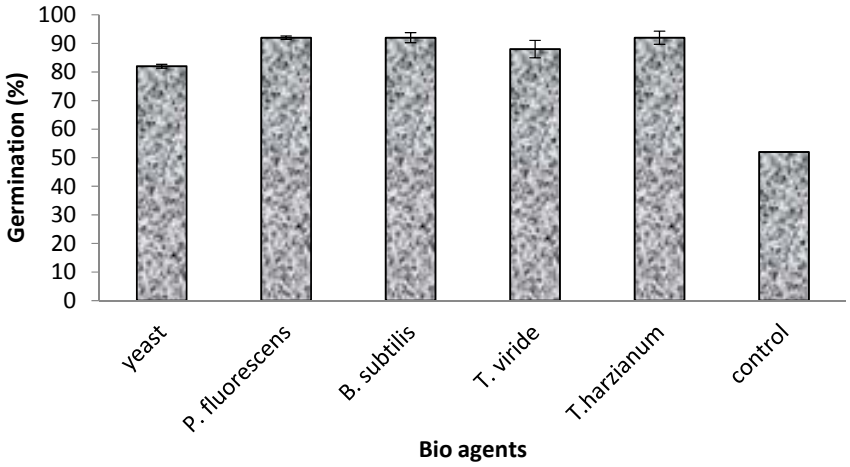


Fig. 4: Effect of different bioagents on germination of sesame seeds. Units are mean ± SE.

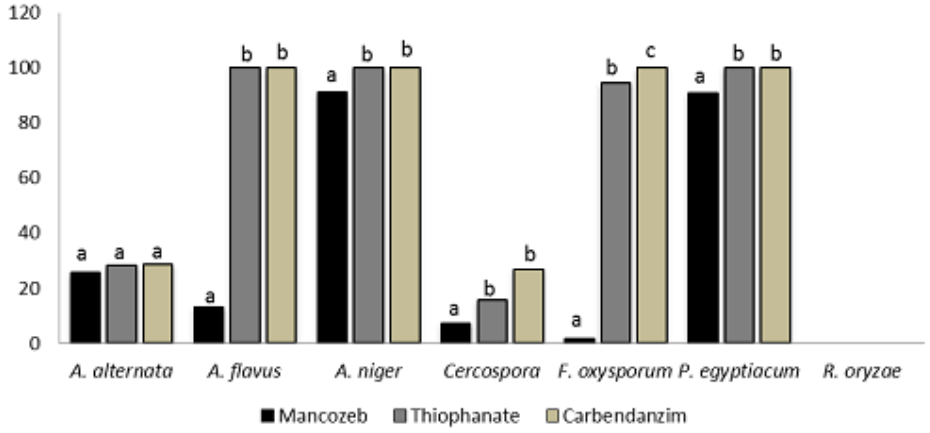


Fig. 5: Effect of three fungicides (0.3 g/kg) on seed pathogens of sesame. Values with different letters show significant difference (P ≤ 0.05) as determined by Tukey's test.

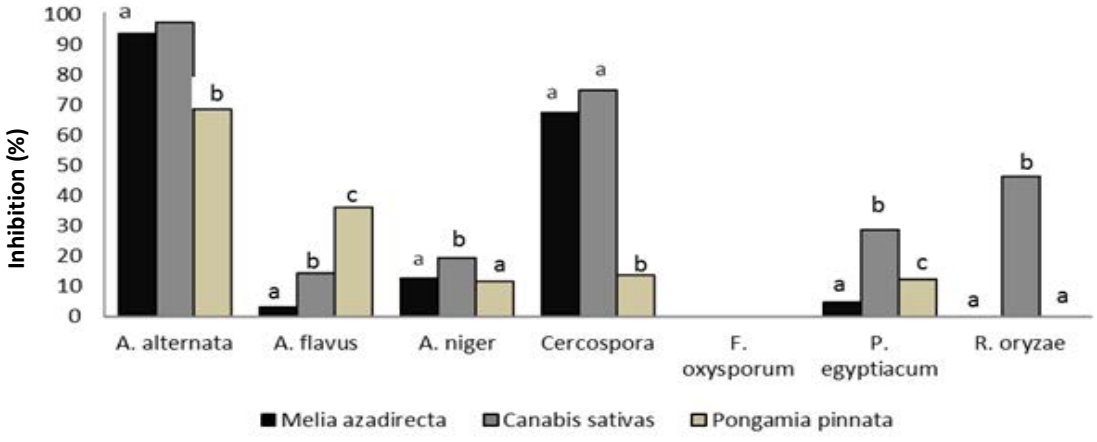


Fig. 6: Effect of plant extracts (10%) on seed pathogens of sesame. Values with different letters show a significant difference (P ≤ 0.05) as determined by Tukey's test.

reduced the incidence of seed-borne fungal pathogens; *T. harzianum* exhibited 86% germination, followed by *T. viride* (82%) and *P. flouorescens* (75%). Mogle and Maske (2012) reported the beneficial effect of *T. viridae* on germination of cowpea seeds.

The most common method of fungal control is the use of chemical fungicides. Fungicide seed treatment is, as often as possible, utilized for enhancing early plant development and controlling the early attack by the pests. This strategy is familiar as useful in diminishing fatalities from seed-borne pathogens and seedling damping off agents (Phipps, 1984; Sinclair and Backman, 1989). Earlier studies also showed that different chemical fungicides exhibited variable effects on seed-borne mycoflora of rice (Ekefan *et al.,* 2006; Thobunluepop *et al.,* 2008). According to the report of Butt *et al.* (2011), a chemical fungicide Antracal completely stopped the growth of *Helminthosporium* sp. and *Curvularia* sp., while topsin, mencozeb and derosal markedly suppressed the growth of *Helminthosporium* by 50%. Topsin and mencozeb also suppressed the growth of *Curvularia* sp., by 50%. Dawar and Ghaffar (1998) reported that seed treatment with fungicide viz. Captan, Batylan, and Benomyl significantly increased seed germination and decreased infection of *A. flavus*, while mancozeb was found most effective in reducing mycelial growth of *F. oxysporum* (Taskeen-Un-Nisa *et al.,* 2011). In another report, Carbendazim (Bavistin) showed complete inhibition of mycelial growth of *Colletotrichum falcatum* (Bhardwaj and Sahu, 2014). Plant extracts showed antifungal activity against a wide range of fungi, therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. The presence of antifungal compounds, in plants, has long been recognized as an important factor in disease resistance (Mahadevan, 1982). The results of this study are consistent with the earlier reports that many plant products contain fungitoxic constituents that have the potential to control plant diseases (Enikuomehin and Peters, 2002). The extracts of *Ocimum gratissimum*, *Azadirecta indica*, and *Mangifera indica* reduced mycoflora load of sesame seeds and germination of extract-treated seeds was higher (78.5-83%) as compared to untreated seeds (72-74%). Ahmed *et al.* (2010) reported aqueous extracts of Majorana, wild chamomile, Geranium oil and Nees plants were highly toxic to seed-borne fungus *Macrophomina phaseolina* isolated from sesame seeds and significantly reduced the mycelial growth of the pathogen. *A. alternata* was inhibited by the extract of *Azadirecta indica* (Al-Hazmi, 2013). Rajani *et al.,* (2012) reported that 73% inhibition of *A. parasiticus* by leaf extract of *Polyanthia longofolia*, while this plant was also reported to be effective in the inhibition of *F. oxysporum* (Dileep *et al.,* 2013). In support of present findings Bharath *et al.* (2005) reported that seed treatment with *T. harzianum* and *T. viride* improved the seed germination, seedling vigour and reduced the incidence of seed-borne fungal pathogens. *T. harzianum* exhibited 86% germination, *T. viride* 82% and *P. flouorescens* 75%. Mogle and Maske (2012) reported the beneficial effect of *T. viridae* on germination of cowpea seeds.

CONCLUSION

The present study highlighted the importance of pre-sowing seed treatments, such as, thermotherapy, fungicides, plant extracts and bioagents in the reduction of mycoflora of sesame seeds. It was observed that seeds treated with fungicides effectively controlled the seed-borne mycoflora but it was not eco-friendly. So, the plant extracts and bioagents can be used as a potential source of herbal fungicides and can be a safe alternative to synthetic chemicals. They increase the seed germination percentage as well as decrease the incidence of seed-borne fungi. Identification of efficient bioagents signifies only the preliminary step toward the development of effective biological control. In order for biocontrol to be implemented on a practical level, the bioagents must be ecologically fit to survive, become established, and function within the particular conditions of the ecosystem. To attain this, much more information regarding the mechanisms of action, ecological characteristics, and interactions with the soil and rhizosphere microbial communities is needed for proper disease management.

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