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Effectiveness of potting media steaming in constraining growth of soil borne pathogenic fungi

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Most of the diseases affecting plant nurseries are soil borne and to eradicate the pathogens from growing media different sterilization (chemical, solar and steam) techniques are used. Steam sterilization is more efficient and environmentally friendly technology. To test the effectiveness of steam sterilization in controlling growth of fungal pathogens, different fungal species were isolated from various potting media (soil, sand, silt, FYM and coconut coir) pre and post steam sterilization and the isolates were characterized. Pre-sterilization fungal isolation was performed using serial dilution method which revealed different fungal species i.e., *Aspergillus flavus*, *A. niger, Fussarium solani*, *Penicillium spp*, *Rhizoctonia spp*, *Curvularia spp,* and *Pythium spp*. After sterilization, one group of potting media was kept open and the other one was tightly packed and placed at ambient temperature. These open and covered media types were evaluated for fungal regrowth by isolation after 7 days, 15 days and 30 days intervals. Different fungal contaminants were found in the potting media that was kept opened after sterilization. However, interestingly no soil related fungal pathogens were observed in the packed potting media 30 days post sterilization. These findings extended the current knowledge of steam sterilization of potting media and suggest that steam sterilization can effectively eliminate the fungal pathogens from the media. Further, it could be effectively used to produce sanitated plant nurseries and minimize the spread of soil borne diseases.
Keywords: Steam sterilization, potting media, fungi, pathogens, soil borne, nursery.

INTRODUCTION

Most of the disease problems in the fruit industry are associated with potting media (soil/soilless) which can be reduced by using good sanitation practices because soil micro-biota contains different types of microbes, fungal spores, weed seeds, nematodes, insects, and some arthropods and these are also liable to thermal death after exposure to higher temperatures (Milbrath, 1930; Hansen *et al*., 2011; Usman and Fatima, 2013). Soil pests spread from one place to another by infected soil media therefore, the use of sterilization of potting media helps in hindering the dissemination of disease-causing agents from nursery to orchards. For partial sterilization of potting media solarization and fumigation can be used. The minimum temperature range for the use of solarization will be 60-70°C exposed for at least 60-30 continuous minutes, respectively. Methyl bromide can also be used for media fumigation in a tight chamber under a plastic sheet at the rate of 2 pounds per 100 cubic feet, however, according to the report of Montreal protocol use of methyl bromide is prohibited for the control of soil borne plant diseases as it causes depletion of Ozone layer (Gay *et al*., 2010; Sharma *et al*., 2011). Fumigation is harmful for human health and is strictly banned (UNEP, 2001). Hence, the eco-friendly sterilization of potting media has been developed because the solarization of potting media may not be applicable in commercial green houses.

Potting media steam sterilization was first time used in USA in 1893. Now-a-days, it is extensively used as an environmentally friendly technology to avoid the spread of pests from fruit plant nurseries to the orchards through infected potting media (Horst and Lawson, 1982). Steam can penetrate more efficiently in the potting media as compared to other sterilization methods like fumigation and heating and effectively kills the soil pests (Hansen *et al*., 2011; Usman and Fatima, 2013) by heating the soil to levels that cause enzyme inactivation and protein coagulation (Quarles, 1997). The plants from these nurseries may be helpful for planting

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orchards of better-quality fruits, yields, long productive life, and can improve income of farmers. Natal Sweet orange trees generated 21% higher production when grafted on healthy Rangpur lime rootstock as compared to Citrus Variegated Chlorosis (CVC) infected plants (Fabricio-Packer *et al*., 2011). Studies revealed that physico-chemical properties i.e., organic matter, pH, EC and moisture retention capacity did not alter by steam sterilization of soil and soilless media and nutrient components like NPK did not alter with the steam sterilization (Elsgaard *et al*., 2010; Usman *et al*., 2013). Using oven for 24 hrs. at 120˚C to sterilized soil and fresh soil similar results were published in Dalbergia (Washa *et al*., 2012) with or without CaO addition (Gelsomino *et al*., 2010). These results propose that there is no hostile effect of steam sterilization of potting media on the nutritional and structural characteristics. Furthermore, the nutritional losses are less in steam sterilization at elevated temperature 80-90˚C for 20-30 min as compared with sterilization for longer intervals of time at 40-60˚C (Usman *et al*., 2013). Steam sterilization is being widely used as an effective way to disinfect damaged compost and potting soil (Quarles, 1997) and has a great impact for commercial nursery growers. It can reduce their production cost by eliminating the potential use of pesticides against the soil borne disease at nursery level and increase the quality of plants by producing healthy nursery plants. Under a USDA funded project sanitation nursery project, the first proto-type steam sterilization unit was developed to sterilize the potting media and develop disease free fruit plants nursery. In this novel study, the steam unit was used for the sterilization of different potting media [field soil, river sand, silt, farmyard manure (FYM), and coconut coir] that have not been studied before for its efficacy in eradicating the soil borne fungal pathogens. In Pakistan, no study related to steam unit efficacy against the soil borne fungal pathogens has been reported. The findings of this study could provide a strong basis for its commercial use in fruit plant nurseries for the development of sanitized nursery plants and controlling soil borne disease dissemination.

MATERIALS AND METHODS

Potting media: Different potting media substrates [field soil, river sand, silt, farmyard manure (FYM), and coconut coir] were collected from the Guava Nursery Sanitation Project, HIS, UAF and kept in the polythene bags. Samples of about 300 g of each potting media were taken and stored in a fridge (4°C) until further processing. The media were steam-sterilized for 30 minutes at ~80°C using a steam sterilization unit (Mistquay International, Faisalabad; Fig. 1).

For this purpose, the steam generator was turned on for the preparation of steam and its pressure (15 psi) was measured on the gauge attached with steam generator. Media was placed in the trolley attached to a steam generator and tightly packed. Steam was allowed to penetrate in the partially moist media with pressure in the trolley for 30 minutes and then steam pipe was disconnected. The media was allowed to cool down and these substrates were stored in three sealable plastic bags (each of 0.6 m^3 size) and used as covered media after 7, 15, and 30 days. Nine samples of each media (around 25 g from each separate media) type were taken and placed at ambient temperature to allow growth of heat-resistant permanent states of soil biota for 30 days in which each three were taken at 7, 15, and 30 days.

Figure 1. Model steam unit for sterilization of potting media. The steam unit includes a steam generator (left) and a media trolley (right) for sterilization of potting media.

***Preparation of Potato Dextrose Agar (PDA)*:** From the fresh potatoes, PDA was prepared according to Abdallah *et al.* (2015). Hundred g of potatoes were peeled, sliced and homogenized with a kitchen blender with 300 mL tap water, allowed to steep overnight at 4 °C, before crude filtration through muslin (potato extract with total volume of 300 mL). A total of 230 mL potato extract, 20 g glucose and 15 g agar were mixed before making up to a final volume of 1 L. For house and commercial preparations, PDA was autoclaved (121 °C/15 min) before dispensing into Petri dishes.

Pre-sterilization fungal isolation from potting media: Potato Dextrose Agar (PDA) media was used for the culturing of fungi (Amadi and Moneke, 2012; MacLowery, *et al*., 1970).

Post-sterilization fungal isolation from potting media: Steam sterilization (eco-friendly media sterilization) method

was used to sterilize potting media. For this purpose, different media were sterilized at 80°C for 30 min. using available steam sterilizer. After sterilization of the potting media, it was evaluated for fungal isolation at different intervals (after 07 days, 15 days, and 30 days) to test re-growth of the microbes.

***Statistical analysis*:** Data were analyzed by one-way analysis of variance (ANOVA) followed by the LSD post hoc test for multiple comparisons by using Statistix 8.1.0. The results are expressed as the mean ± standard error as bar and were considered statistically significant at $p < 0.05$.

RESULTS

Pre-sterilization fungal isolation from different soil and soilless media: After the first incubation colonies of different colors and shapes appeared on the PDA plate from the field soil samples, was sub-cultured and maintained on PDA plate and slants. Six single colonies (FS-1, FS-2, FS-3, FS-4, FS-5, and FS-6) were successfully isolated and purified (Table 1 and Fig. 2). These isolates were morphologically identified as *Aspergillus flavus*, *A. niger*, *Unidentified*, *Rhizoctonia spp.*, *Fusarium solani*, and *Penicillium spp.*, respectively. *Aspergillus flavus* was found significantly high (35%) followed by *Rhizoctonia spp*. (18%; Fig. 3A).

Four single colonies (RS-1, RS-2, RS-3, and RS-4) were also successfully purified from river sand media (Fig. 1 and Table 1) using morphological characteristics and were identified as *Aspergillus flavus*, *Penicillium spp.*, *Rhizoctonia spp.*, and *Curvularia spp.*, respectively. Moreover, *Curvularia spp*, was found significantly high (40%) followed by *Penicillium spp*. (30%; Fig. 3B).

Table 1. Characterization of fungal isolates detected from different potting media

Source	Colony characters		Diameter	Colony color		Mycelium Characters			Conidia Characters		
	Isolates	Texture	(mm) *	Dorsal	Ventral	Type	Septation	Color	Size (µm)	Shape	Spores type
Field Soil											
FS-1	*Aspergillus flavus*	Velvety	4.5	Green	Yellowish	Branched	Septate	Hyaline	3.5-5	Spherical-oval	Conidia
FS-2	*Aspergillus niger*	Woolly	4.6	Black	Black	Branched	Septate	Hyaline	4-6	Spherical	Conidia
FS-3	*Unidentified*	Compact	3.3	White	White	Branched	Septate	Hyaline	4-7.5	Spherical	Conidia
FS-4	*Rhizoctonia spp.*	Sparse	3.4	Off white/creamy	Yellowish	Branched	Septate	Pale white	1000-3000	Irregular	Sclerotia
FS-5	*Fusarium solani*	Cottony	3	White	Yellow	Branched	Septate	White cream	9.4 x 3.6	Curved/ Crescent	Macro-Conidia
FS-6	*Penicillium spp.*	Velvety	3.5	Grayish	Blackish	Branched	Septate	Grayish green	2.8–3.5	Globose	Conidia
River Sand											
RS-1	*Aspergillus flavus*	Compact	3.9	Green	White	Branched	Septate	Hyaline	3.5 – 5	Spherical-oval	Conidia
RS-2	*Penicillium spp.*	Velvety	3	Grayish	Blackish	Branched	Septate	Hyaline	2.8 – 3.5	Globose	Conidia
RS-3	*Rhizoctonia spp.*	Sparse	3.4	Off white/creamy	Yellowish	Branched	Septate	Pale white	1000-3000	Irregular	Sclerotia
RS-4	*Curvularia spp.*	Compact	3.2	Brown	Dark brown	Branched	Septate	Dark brown	13 - 29	Curved	Conidia
Silt											
S-1	*Aspergillus flavus*	Velvety	4.1	Green	Yellow	Branched	Septate	Hyaline	3.5 – 5	Spherical-oval	Conidia
S-2	*Penicillium spp.*	Velvety	3.9	Grayish	Blackish	Branched	Septate	Hyaline	2.8 – 3.5	Globose	Conidia
S-3	*Rhizoctonia spp.*	Compact fluffy	3.1	Off white	Creamy	Branched	Septate	Whitish	1000-3000	Irregular	Sclerotia
S-4	*Curvularia spp.*	Compact	3	Brown	Dark Brown	Branched	Septate	Deep brown	13 - 29	Curved	Conidia
S-5	*Pythium spp.*	Filamentous	3.2	White	White	Branched	Septate	Whitish	20–22.5	Globose ellipsoid	Oospore
FYM											
FM-1	*Aspergillus flavus*	Velvety	3.8	Green	Yellow	Branched	Septate	Hyaline	3.5-5	Spherical-oval	Conidia
FM-2	*Aspergillus niger*	Woolly	3.5	Black	Black	Branched	Septate	Hyaline	6-4	Spherical	Conidia
FM-3	*Penicillium spp.*	Velvety	3.6	Gray	Blackish	Branched	Septate	Hyaline	2.8–3.5	Globose	Conidia
FM-4	*Haline mycelim*	Compact	3.1	White	White	Branched	Septate	Hyaline	4.1 – 5	Oval	Conidia
FM-5	*Curvularia spp.*	Compact	3	Brown	Blackish	Branched	Septate	Dark Brown	13 - 29	Curved	Conidia
Coco-Coir											
CC-1	*Aspergillus flavus*	Velvety	3.5	Dark green	Blackish	Branched	Septate	Hyaline	3.5-5	Spherical-oval	Conidia
CC-2	*Penicillium spp.*	Velvety	4	Grayish	Blackish	Branched	Septate	Hyaline	2.8–3.5	Globose	Conidia
CC-3	*Aspergillus niger*	Wooly	3.5	Black	Black	Branched	Septate	Hyaline	4 – 6	Spherical	Conidia

*Diameter of colony measured after 7 days of inoculation

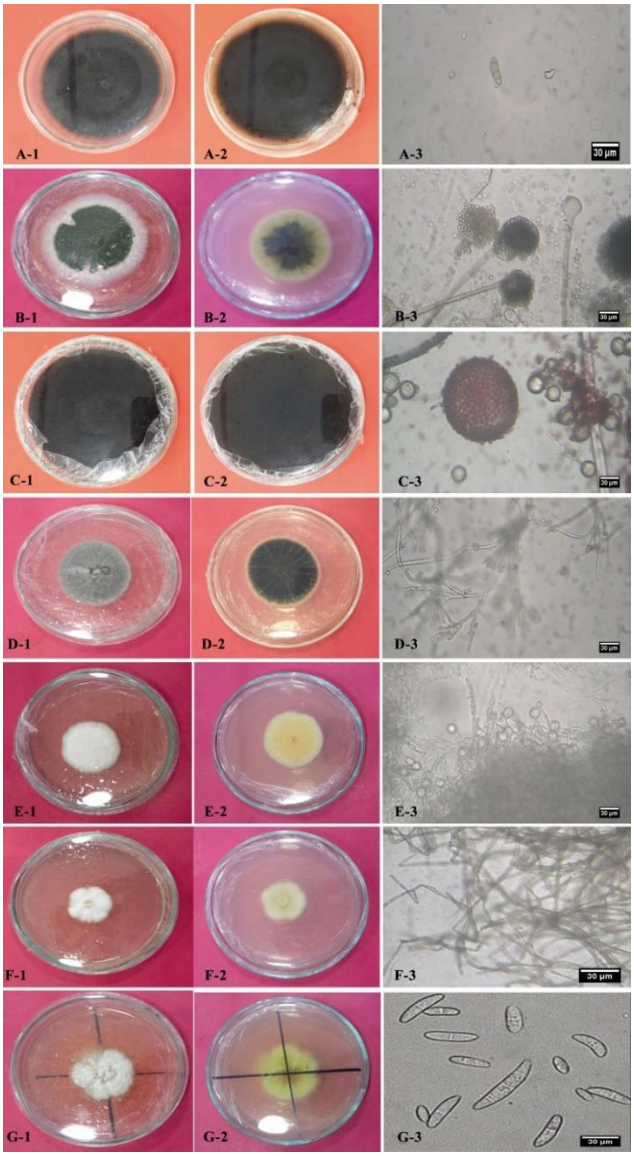

Figure 2. The fungal isolates identified pre-sterilization of potting media.

A-1: dorsal view of colony plate *Curvularia spp.*, **A-2:** ventral view of the colony plate *Curvularia spp.*, and **A-3:** microscopic picture of *Curvularia spp*. **B-1:** dorsal view of colony plate *Aspergillus flavus*, **B-2:** ventral view of the colony plate *Aspergillus flavus*, and **B-3:** microscopic picture of *Aspergillus flavus.* **C-1:** dorsal view of colony plate *Aspergillus niger*, **C-2:** ventral view of the colony plate *Aspergillus niger*, and **C-3:** microscopic picture of *Aspergillus niger.* **D-1:** dorsal view of colony plate *Penicillium spp.*, **D-2:** ventral view of the colony plate *Penicillium spp.*, and **D-3:** microscopic picture of *Penicillium spp.* **E-1:** dorsal view of colony plate *Pythium spp.*, **E-2:** ventral view of the colony plate *Pythium spp.*, and **E-3:** microscopic picture of *Pythium spp.* **F-1:** dorsal view of colony plate *Rhizoctonia spp.*, **F-2:** ventral view of the colony plate *Rhizoctonia spp.*, and **F-3:** microscopic picture of *Rhizoctonia spp.* **G-1:** dorsal view of colony plate *Fusarium solani*, **G-2:** ventral view of the colony plate *Fusarium solani*, and **G-3:** microscopic picture of *Fusarium solani.*

Five single colonies (S-1, S-2, S-3, S-4, and S-5) were successfully isolated from silt media (Figure 2 and Table 1) and were morphologically identified as *Aspergillus flavus*, *Penicillium spp.*, *Rhizoctonia spp.*, *Curvularia spp.* and *Pythium spp.* respectively. In which *Rhizoctonia spp.*, was found most abundant (35%) followed by *Penicillium spp.* (20%) and *Pythium spp* (20%; Fig. 3C).

Five single colonies (FM-1, FM-2, FM-3, FM-4, and FM-5) were successfully isolated from FYM media (Figure 2 and Table 1) were identified as *Aspergillus flavus, Aspergillus niger, Penicillium spp,* un-identified (hyaline mycelium) and *Curvularia spp,* respectively. In which *Curvularia spp,* was found significantly high (30%) and *Penicillium spp* was significantly low (8%) (Fig. 3D).

Three single colonies (CC-1, CC-2, and CC-3) were successfully isolated from coconut coir media on new plates (Figure and Table) and were identified as *Aspergillus flavus, Penicillium spp,* and *Aspergillus niger* respectively. *Penicillium spp,* was found significantly high (40%) followed by *Aspergillus flavus* (35%; Fig. 3E). All the identifications were performed according to the protocols as explained previously (Booth, 1971; Ellis, 1971; Barnett and Hunter, 1972).

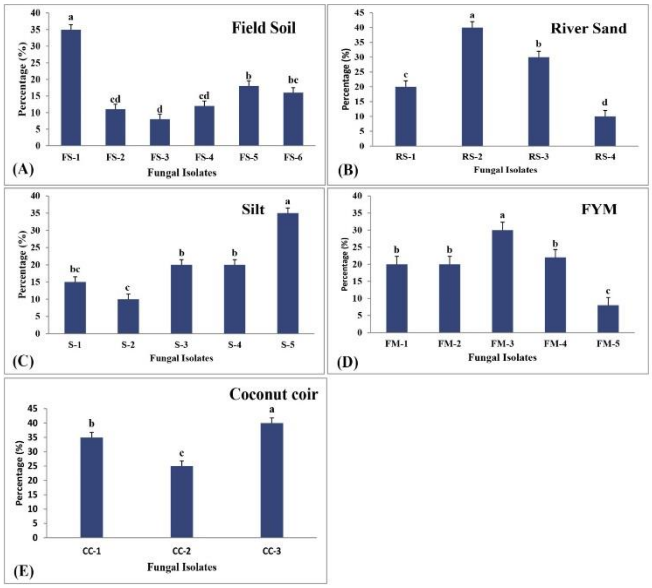

Figure 3. Percentages of different fungal isolates taken from different potting media.

A: represent the percentage of fungal isolates from field soil. **B**: represent the percentage of fungal isolates from river sand. **C**: represent the percentage of fungal isolates from silt. **D**: represent the percentage of fungal isolates from FYM. **E:** represent the percentage of fungal isolates from coconut coir. Error bars indicate the standard error as mean + SD and Bars affected with the same letter are not significantly different according to LSD test at $P \leq 0.05$.

After steam sterilization of potting media, the media was divided into two groups as covered and uncovered, In the

covered field soil sample, *A. niger* and *A. flavus* were found after 7, 15, and 30 days of steam sterilization. In the uncovered field soil after the steam sterilization, *A. flavus, Penicillium spp.*, and *A. niger* were found from 7, 15, and 30 days of treatment (Table 2). In the covered river sand sample, *A. niger* was identified after 15, and 30 days of steam sterilization. In the uncovered field soil after the steam sterilization *Penicillium spp.*, and *A. niger* were found from 15, and 30 days of treatment (Table 2). In the covered silt sample, *A. flavus* was identified after 15, and 30 days of steam sterilization but in the uncovered field soil after the steam sterilization *Penicillium spp.*, and *A. flavus* were found after 7, 15, and 30 days of treatment. For the FYM potting media, there was no fungal isolate found after 24 hours both in the covered and uncovered media. In the covered FYM sample, *Penicillium spp.* Was identified after 7, 15, and 30 days of steam sterilization but in the uncovered field soil after the steam sterilization *Penicillium spp.*, and *A. niger* were found from 7, 15, and 30 days of treatment (Table 2). For the coconut coir potting media, there was no fungal Isolate found 7, 15, 30 days in covered sample. In the uncovered field soil sample, *Penicillium spp.* was identified after 15, and 30 days of steam sterilization (Table 2).

Table 2. Isolation and identification of fungal isolates from potting media post steam sterilization.

Media	Duration after sterilization (days)	Covered media	Uncovered Media
Field Soil			
	7	No	*Aspergillus niger*
	15	*Aspergillus flavus*	*Penicillium spp.*
	30	*Aspergillus flavus*	*Aspergillus niger* *Penicillium spp.*
River Sand			
	7	No	No
	15	No	*Penicillium spp.*
	30	No	*Penicillium spp.*
Silt			
	7	No	No
	15	*Aspergillus flavus*	*Aspergillus flavus*
	30	*Aspergillus flavus*	*Aspergillus flavus*
FYM			
	7	*Penicillium spp.*	*Aspergillus niger*
	15	*Penicillium spp.*	*Penicillium spp.*
	30	*Penicillium spp.*	*Aspergillus niger* *Penicillium spp.*
Coco Coir			
	7	No	No
	15	No	No
	30	No	*Penicillium spp.*

DISCUSSION

Potting media sterilization provides healthy plant material for orchards with good quality production, high yield and prolonged productivity than the substandard and infected plants (Fabricio-Packer *et al.*, 2011; Hyun *et al.*, 2023). Different methods have been used for the treatment of potting media infected with various microorganisms' viz. pasteurization at 63°C with aerated steam, chemical sterilization with methyl bromide, and at 93°C steam sterilization to leave certain saprophytic harmful organisms in the media (Allen and Lamb, 1981; Dietrich *et al.*, 2020). For dry and dry climates steaming of media is accepted (van Leonen *et al.*, 2003). Compared to soil fumigation and dry heating, steam penetrates more efficiently into the media, is more effective to control soil pests (Hansen *et al.*, 2011). It was found that steam sterilization of media up to 60°C for 30 minutes may eliminate pathogens such as fungi, bacteria nematodes and viruses (Baker, 1967). At certain temperature and pressure steam sterilization is applied for the eradication of potential pathogens present in the soils. Eggs of different insects present in the potting media can also be killed by steam sterilization. When soil media is steam sterilized at 90-121ºC temperature and 15 Psi it also deactivates the soil pathogens (Wang *et al.*, 2011). In this study, five different types of potting media (field soil, river sand, silt, FYM, and coconut coir) were used to assess the efficacy of steam sterilization. Different fungal species including plant pathogenic fungi i.e., *A. flavus, A. niger, Rhizoctonia spp., F. solani, Penicillium spp., Curvularia spp.,* and *Pythium spp.* were identified before the steam sterilization. Fifteen fungal species from agricultural fields belonging to six genera were isolated by Gaddeyya et al., (2012) at Salur Mandal, India (*Fusarium spp., Rhizopus sp*., *Aspergillus spp*., *Trichoderma spp*., *Penicillium spp*., and *Curvularia spp.*) by using soil dilution technique and soil plate technique. *Aspergillus flavus, A. nidulans, A. niger, A. fumigatus, A. terreus, Penicillium chrysogenum,* and *P. frequentens* were isolated from the soil samples from different rice fields at Tekkali region of Srikakulam (Kumar *et al*., 2015). Fungal species belonging to the genera *Aspergillus*, *Colletotrichum*, *Cunninghamella*, *Cladophialophora Cunninghamella*, *Mucor*, *Penicillium*, *Rhizopus*, and *Scopulariopsis* were detected from soil samples taken from 20 zones in Chennai, through soil dilution method besides staining technique by using cotton blue and Lactophenol (Raja et al.,2017). Seventy-seven samples collected from coastal regions of Red Sea, *Aspergillus*, *Alternaria*, *Cladosporium*, *Emericella*, *Fusarium*, *Penicillium*, *Thielavia*, and *Scytalidium* were detected, and the identification was done by using both phenotype and genotype techniques (Alwakeel, 2017).

In this research work, it was found that when different potting media were steam sterilized at 15 Psi pressure and 80°C temperature for 30 minutes, there were few non-pathogenic fungal isolates found 17 days post-sterilization, however, from the uncovered potting medium a few more fungal isolates were found. Isolation of fungi from uncovered potting medium revealed that these fungal cultures may have developed from the potting media because *Aspergillus* species

are considered among the potential thermo-tolerant antagonists surviving after soil solarization such as *A. niger, A. flavus, and A. fumigatus* (Tjamos and Paplomatas, 1987). Recently, many atoxigenic *Aspergillus spp.* strains isolated from soil were used as an effective biocontrol against *Aspergillus* toxigenic strains (Degola *et al*., 2011). More likely these could be contaminants because of their high percentage in the environment and most of the plant fungal pathogens are not very heat resistant (Endlweber and Scheu 2006; Luo, *et al*., 2022). *Penicillium* species are ubiquitous soil fungi; different studies have revealed that *Penicillium* spp. enhances the plant growth by their interaction with roots of crop plants (Shivanna *et al*. 1994; Khan *et al*. 2008). Some *Penicillium* species are famous for the production antibiotics through which they activate the multiple defense signals and induce resistance in plants (Hossain *et al*. 2007). They may help the plant to grow in salt stress conditions and withstand different environmental stress conditions (Khan *et al*. 2011). Interestingly most of the important soil borne pathogenic fungi such as *Fusarium solani, Curvularia spp,* and *Pythium spp*. was not found post steam sterilization of the potting media. The variation in the pathogenicity level of *Fusarium* spp. has been revealed in different studies (Gachango *et al*. 2012; Gashgari and Gherbawy 2013), the genus *Curvularia and Pythium* have different pathogens and saprobes of different plants (Manamgoda *et al*., 2011; Schroeder *et al*., 2013). Our study shows that steam sterilization changed the biotic properties of different potting media, which is in line with studies on the effects of autoclaving (Trevors 1996, Berns *et al*. 2008). Regarding efficiency of steam sterilization, it is recommended to perform steam-sterilization treatments to reduce the potting media biota especially because mycorrhizae are not very heat-resistant (Endlweber and Scheu 2006).

Conclusions: Our study highlights that steam sterilization successfully eliminated most of the pathogenic fungal species from different types of potting media. In this novel study, different potting (soil, sand, silt, FYM and coconut coir) media components were steam sterilized and evaluated against the soil borne fungal pathogens and found that steam sterilization is an efficient, cost effective and environment friendly alternative to other media sterilization methods, especially when large amount of soil substrate is required for commercial nurseries. Therefore, utilization of steam sterilization technology is recommended for elimination of the fungal pathogens in commercial nurseries. Furthermore, this technology may also be used to kill soil borne pathogens in the beds of vegetable growing tunnels, promote organic vegetable production and minimize the use of toxic pesticides.

***Conflict of Interest*:** The authors declare that there is no conflict of interest.

***Author's Contribution Statements*:** FUR and MHS executed the work in field, KPA supervised the work in Nuclear Institute for Agriculture and Biology (NIAB), whereas MU and BF conceived the idea and supervised the whole research work, MU provided funds for research and edited the article.

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REFERENCES

A.L. Khan, M. Hamayun, Y.H. Kim, S.M. Kang and I.J. Lee. 2011. Ameliorative symbiosis of endophyte (*Penicillium funiculosum* LHL06) under salt stress elevated plant growth of Glycine max L. Plant Physiology and Biochemistry 49:852-861.

Allan, P., D. Lamb and D. Chalton. 1981. Sterilization and pasteurization of soil mixes. S. African Avocado Growers Association. Yearbook 4:124-127.

Alwakeel, S.S. 2017. Molecular identification of fungi isolated from coastal regions of Red Sea, Jeddah, Saudi Arabia. The Association of Arab Universities for Basic and Applied Sciences 24:115-119.

Amadi O.C. and A.N. Moneke. 2012. Use of starch containing tubers for the formulation of culture media for fungal cultivation. African Journal of Microbiology Research 6:4527-4532.

Aydi Ben Abdallah R, Jabnoun-Khiareddine H, Mejdoub-Trabelsi B, Daami-Remadi M 2015. Soil-borne and Compost-borne Aspergillus Species for Biologically Controlling Post-harvest Diseases of Potatoes Incited by Fusarium sambucinum and Phytophthora erythroseptica. J Plant Pathol Microbiol 6:313.

Baker, K.F. 1967. Control of soil-borne plant pathogens with aerated steam. Proc. Wash. State Univ. Greenhouse Growers Institute pp. 3-18.

Barnett, H. L., and B. B. Hunter. 1972. Illustrated genera of imperfect fungi. Illustrated *genera of imperfect fungi.*, (3rd ed).

Berns, A., H. Philipp, H.D. Narres, P. Burauel, H. Vereecken and W. Tappe. 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. European Journal of Soil Science 59: 540–550.

Booth, C. 1971. *The genus Fusarium*. Kew, UK, Commonwealth Mycological Institute.

Degola F., E. Berni, and F.M. Restivo. 2011. Laboratory tests for assessing efficacy of atoxigenic *Aspergillus flavus* strains as biocontrol agents. International Journal of Food Microbiology 146: 235-243.

Dietrich, P., Cesarz, S., Eisenhauer, N., & Roscher, C. 2020. Effects of steam sterilization on soil abiotic and biotic properties. Soil Organisms 92: 99-108.

Ellis, M. B. 1971. Dematiaceous hyphomycetes. *Dematiaceous hyphomycetes.*

Elsgaarda, L., M.H. Jørgensenb and S. Elmholt. 2010. Effects of band-steaming on microbial activity and abundance in organic farming. Soil Agriculture Ecosystems and Environment 137: 223-230.

Endlweber, K. and S. Scheu. 2006. Establishing arbuscular mycorrhiza-free soil: a comparison of six methods and their effects on nutrient mobilization. Applied Soil Ecology 34:276–279.

Fabrício-Packer, G.A., S. Eduardo-Sanches, D.S. Simone-Rodrigues, R. Eduardo-Toller and A. Lilian. 2011. Role of healthy nursery plants in orange yield during eight years of Citrus Variegated Chlorosis epidemics. Scientia Horticulturae 129:343-345.

Gachango E., L. Hanson, A. Rojas, J. Hao and W. Kirk. 2012. Fusarium spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Disease 96:1767–1774.

Gaddeyya, G., Niharika, P. S., Bharathi, P. and P.K.R. Kumar. 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Advances in Applied Science Research 3:2020-2026.

Gashgari R.M., Y.A. Gherbawy. 2013. Pathogenicity of some Fusarium species associated with superficial blemishes of potato tubers. Polish Journal of Microbiology 62:59–66.

Gay, P., P. Piccarolo, D.R. Aimonino and C. Tortia. 2010. A high efficiency steam soil disinfestation system, part I: Physical background and steam supply optimization. Biosystems Engineering 107:74-85.

Gelsomino, A., B. Petrovi_cová, F. Zaffina and A. Peruzzi. 2010. Chemical and microbial properties in a greenhouse loamy soil after steam disinfestation alone or combined with CaO addition. Soil Biology and Biochemistry 42:1091-1100.

Hansen, J.D., J.A. Johnson and D.A. Winter. 2011. History and use of heat in pest control. A review. International Journal of Pest Management 57:267-289.

Horst, R.K. and R.H. Lawson. 1982. Soil sterilization: an economic decision. Greenhouse Manager 99: 55-61.

Hossain M.M., F. Sultana, M. Kubota, H. Koyama and M. Hyakumachi. 2007. The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiology 48:1724-1736.

Hyun, J. E., Lee, S. B., Jung, D. Y., Kim, S. R., Choi, S. Y., & Hwang, I. 2023. Effects of sterilization methods on the survival of pathogenic bacteria in potting soil stored at various temperatures. Food Science and Biotechnology 32:111-120.

Khan S.A., M. Hamayun, H. Yoon, H.Y. Kim, S.J. Suh, S.K. Hwang, J.M. Kim, I. J. Lee, Y.S. Choo and U.H. Yoon. 2008. Plant growth promotion and *Penicillium citrinum*. BMC Microbiology 8:1-10

Kumar, P. K. R., G. Hemanth, P.S. Niharika and S. Kolli. 2015. Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District. International Journal of advances in Pharmacy, Biology and Chemistry 4:484-490.

Luo, Y., van Veelen, H. P. J., Chen, S., Sechi, V., ter Heijne, A., Veeken, A., ... & Bezemer, T. M. 2022. Effects of sterilization and maturity of compost on soil bacterial and fungal communities and wheat growth. *Geoderma 409*: 115598

MacLowery, J.D., M.J. Jaqua and S.T. Selepak. 1970. Detailed Methodology and Implementation of a Semiautomated Serial Dilution Microtechnique for Antimicrobial Susceptibility Testing. Applied and Environmental Microbiology 20:46-53.

Manamgoda D.S., A.Y. Rossman, L.A. Castlebury, E. Chukeatirote and K.D. Hyde. 2015. A taxonomic and phylogenetic re-appraisal of the genus *Curvularia* (Pleosporaceae): human and plant pathogens. Phytotaxa 212:175–198.

Milbrath, D.G. 1930. Treatment of soil for prevention of plant diseases. Calif Dep Agric Mon Bull 18:7-15.

Quarles, W. 1997. STEAM - The Hottest Alternative to Methyl Bromide. American Nurseryman 37–43.

Raja, M., G. Praveena and S.J. William. 2017. Isolation and Identification of Fungi from Soil in Loyola. Current Microbiology and Applied Sciences 6:1789-1795.

Schroeder, K.L., F.N. Martin, A.W.A.M. de Cock, C.A. Lévesque, C.F.J. Spies and P.A. Okubara. 2013. Molecular detection and quantification of *Pythium* species: evolving taxonomy, new tools, and challenges. Plant Disease 97:4-20.

Sharma, S.D., P. Kumar, S.K. Bhardwaj and A. Chandel. 2011. Symbiotic effectiveness of arbuscular mycorrhizal technology and Azotobacterization in citrus nursery production under soil disinfestation and moisture conservation practices. Scientia Horticulturae 132:27-36.

Shivanna M.B., M.S. Meera and M. Hyakumachi. 1994. Sterile fungi from zoysiagrass rhizosphere as plant growth promoters in spring wheat. Canadian Journal of Microbiology 40:637–644.

Tjamos E.C. and E.J. Paplomatas. 1987. Effect of soil solarization on the survival of fungal antagonists of *Verticillium dahliae*. EPPO Bull 17:645-653.

Trevors, J.1996. Sterilization and inhibition of microbial activity in soil. Journal of Microbiological Methods 26:53–59.

UNEP. 2001. Report of the Technology and Economic Assessment Panel. United Nations Environmental Program, Nairobi, Kenya p. 74.

Usman, M. and B. Fatima. 2013. Nursery sanitation in Citrus and Guava: A way forward. p. 1-22. In: M. Usman and B. Fatima (ed.). Citrus and Guava Nursery Raising: A practical approach. PAK TM publishers Faisalabad, Pakistan.

Van Loenen, M.C.A., Y. Turbett, C.E. Mullins, N.E.H. Feilden, M.J. Wilson, C. Leifert and W.E. Seel. 2003. Low temperature-short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds. European Journal of Plant Pathology 109:993-1002.

Wang, C.Y., F. Wang, T. Wang, X.L. Yang, Y.R. Bian, F.O. Kengara Z.B. Li and X. Jiang. 2011. Effects of autoclaving and mercuric chloride sterilization on PAHs dissipation in a two-liquid-phase soil slurry. Pedosphere 21:56-64.

Washa, W. B. A., A. M. S. Nyomora, and H. M. V. Lyaruu. 2012. Improving propagation success of D. melanoxylon (African blackwood) in Tanzania (II): rooting ability of stem and root cuttings of Dalbergia melanoxylon (African blackwood) in response to rooting media sterilization in Tanzania. Tanzania Journal of Science 38: 43-53.