Formalin fumigation and steaming of various composts differentially influence the nutrient release, growth and yield of muskmelon (*Cucumis melo* L.)

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Nutrient disorder and presence of disease-causing agents in soilless media negatively influence the growth of muskmelon. To combat these issues, use of environmentally-friendly sanitation techniques is crucial for increased crop productivity. The study was conducted under greenhouse and field conditions to investigate the effect of two different sanitation techniques: steaming and formalin fumigation on various media's characteristics and their impact on muskmelon yield. Media: jantar, guar, wheat straw and rice hull and peat moss of 10% air-filled porosity and sanitized with formalin and steaming. Steaming of guar, jantar, and wheat straw increased the phosphorus (P) and potassium (K) concentrations by 13.80–14.86% and 6.22–8.45% over formalin fumigation. Likewise, P and K concentrations in muskmelon were higher under steaming. Steaming significantly inhibited the survival of *Fusarium* wilt sp. *melonis*, root knot nematode sp. *meloidogyne* and nitrifying bacteria in media than formalin fumigation. In conclusion, steaming decreased the prevalence of nitrifying bacteria and pathogens which thus improved the NO3−–N:NH4+–N ratios, P and K nutritional balance both in the media and muskmelon transplants. Hence, steaming as an environment-friendly approach is recommended for soilless media. Further, optimization of steaming for various composts with different crops needs to be investigated with steaming technique.

Plant based-media have a great potential as an alternative to peat moss. It may limit the use of soils and reduce the fertilizer cost for transplant growth1. Farmers can also earn an additional income by marketing their own

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peat alternatives and sanitation techniques should fulfill media’s health requirements. In addition, the AFPs of guar, jantar, and wheat straw were significantly influenced by the sanitation techniques, but remained in the suitable range for the growth of muskmelon.

Chemicals may also reduce the severity of both soil and seed-borne diseases. For instance, soil fumigation with metam sodium decreased the potato scab by 98%. Ammonia gas fumigation in the cucumber field suppressed the harmful plant pathogen causing Fusarium wilt disease and enhanced the yield. Likewise, dimethyl sulphide was an effective soil fumigant against nematodes. Other than chemicals, steam disinfection, soil solarization, and biochar application are effective. Disinfection techniques kills nematodes and pathogens, however may harm beneficial microbes and have negative impacts on the plant growth and development.

Agricultural wastes must be considered as an attractive source for making value-added products in order to address food security challenges. In this regard, guar, jantar, wheat straw and rice hull are abundant agricultural wastes that can be utilized and processed through composting. Guar and jantar being leguminous in nature, have low nutrient requirements for their production, commercially produced and may be utilized for N source of fertilization.

Muskmelon is an important vegetable crop from Cucurbitaceae family cultivated throughout the world. Muskmelon is a short duration crop and can respond quickly to the nutrient supply while raising muskmelon transplants.

Results

Germination, mortality and muskmelon yield. Interactive effects of media and sanitation techniques were significant for plant growth traits like seed germination, root length, leaf chlorophyll contents, number of leaves, leaf area, stem thickness and shoot fresh weights (Table 1). The generalized ANOVA exhibited significance (F-value) for root length (29.37), no. of leaves per seedling (468.69), leaf area (6.94) and shoot fresh weight (3.49) at p ≤ 0.05, 0.01 and 0.001 levels. Overall, steaming of the composts performed better for growth rates and yield among sanitation treatments followed by formalin fumigation and unsanitized control (lowest yield). Rice hull showed the best growth and yield among composts (Fig. 1a–c).

Similar to growth, muskmelon yield was positively influenced by steaming of the composted media (Fig. 1d–f). Muskmelon transplants grown under rice hull compost had maximum yield (3.50–3.25 kg plant⁻¹). At all sites, the media under the influence of steaming increased the yield plant⁻¹ than transplants grown in media receiving formalin fumigation.

Establishing AFP of the composts. Results of the present study revealed that particle sizes greatly influenced the AFPs of the media (Tables S1 and S2). Relative proportions of particles of 2–3.3 mm and < 2 mm were associated with AFPs of guar (R² = 0.94), jantar (R² = 0.83) and wheat straw (R² = 0.91). In the case of rice hull compost, relationship between varying proportions of particle sizes of 1–2 mm blended with 0.5–1 mm and AFPs was highly significant (R² = 0.88).

Physicochemical properties of the sanitized media. Electrical conductivity (EC) and pH were not significantly influenced by the sanitation techniques, but remained in the suitable range for the growth of muskmelon (Table S3). Although all the media were of same AFP levels, water holding capacity (WHC) of the composts varied.

Population of nitrifying bacteria and nitrogen transformation in the media and muskmelon transplants. Sanitation reduced the population of nitrifying bacteria in the media at 0 and 10 d when compared with the control and fumigation, respectively. Nitrifying bacteria got recovered after 20 and 30 d of sanitation, however, population of these bacteria was high 30 d after sanitation of the media (Fig. 2a). In general, steaming sanitation had lower population of nitrifying bacteria than formalin fumigation.

Nitrate (NO₃−N) concentrations in all the steam sanitized media except peat moss at 0 and 10 d were increased over formalin fumigated (Fig. 2b). Similarly, ammonium (NH₄⁺–N) concentrations were increased by steaming of guar and rice hull than formalin fumigation (Fig. 2c). Steaming of the media increased the NO₃−N concentrations at 20 d and 30 d as compared to formalin fumigation. In contrast to NO₃−N, NH₄⁺–N concentrations in the media after 20 d and 30 d of sanitation varied largely among the composts. Moreover,
NO₃⁻–N + NH₄⁺–N in concentrations in the steam sanitized media were higher than that of formalin-fumigation (Fig. 2d). Nitrate–N and NH₄⁺–N concentrations were accumulated more in muskmelon transplants which were grown in sanitized media (Fig. 3).

Table 1. Estimates of least square means ± standard errors calculated on account of natural log (on the scale of inference) with their exponential means (on the scale of measurement) for the determination of experimental variables and their probabilities of germination. Means sharing similar letter in a row or in a column are statistically non-significant (p ≤ 0.05).

Figure 1. Effect of composts and sanitation techniques on: (a) growth rate (cm week⁻¹) for site 1; (b) growth rate (cm week⁻¹) for site 2; (c) Growth rate (cm week⁻¹) for site 3; (d) yield (kg plant⁻¹) for site 1; (e) yield (kg plant⁻¹) for site 2, (f) yield (kg plant⁻¹) for site 3 of muskmelon. All the values are means ± S.E of three replicates, whereas case letters indicate significant differences among the treatments at p ≤ 0.05 level.
**Figure 2.** Effect of sanitized media on: (a) relative abundance of nitrifying bacteria over the time, (b) NO$_3^-N$ mg kg$^{-1}$ media, (c) NH$_4^+-$N mg kg$^{-1}$ media and (d) NO$_3^-N$ + NH$_4^+-$N mg kg$^{-1}$ media over the time. All the values are means ± S.E of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level. All the composts used in greenhouse experiment were established at 10% AFP. DAS = Days after sanitation.

**Nutrient concentrations of the media and muskmelon transplants as influenced by sanitation.** Similar to N-forms, total N concentrations in the steam sanitized media were increased (Fig. 4a). Steam sanitized media increased the P concentrations of guar, jantar and wheat straw by 14.86%, 13.80% and 14.24% greater as compared to the formalin fumigation (Fig. 4b). Similarly, K concentrations in steam sanitized guar, jantar, and wheat straw composted media were increased by 6.22%, 7.54% and 8.45% when compared with the formalin fumigation (Fig. 4c).

Steaming increased the P concentrations in muskmelon transplants grown under peat, guar, jantar, wheat straw, rice hull composted media by 9.97%, 13.03%, 13.59%, 17.25% and 3.25% when compared with the formalin fumigation (Fig. 4d). Similarly, K concentrations in steam sanitized guar, jantar, wheat straw and rice hull media were 9.62%, 4.32%, 2.61%, 6.82% and 5.26% higher than the transplants received formalin fumigation (Fig. 4c).

**Prevalence of root knot nematodes and Fusarium wilt.** Between the two sanitation techniques, steaming significantly reduced the disease severity of root knot nematodes and *Fusarium* wilt (Fig. 5 and Table 3). Moreover, steaming sanitation inhibited the population of disease-causing agents in all the media.

**Discussion**

Seed germination and root length are the foremost indicators contributing to plant health and survival. In the present study, higher germination% in the rice hull compost was probably due to non-woody nature of the compost which thus decomposed when sanitized and enhanced seed germination. Rice hull compost provides optimal concentrations of N, P, K and additional supplements like Si, which thus improve the plant growth and development. Moreover, the increase in seed germination and root length of steam sanitized media over their corresponding controls was correlated with the increased nutrient availability. Steaming of the media probably influenced the C:N ratio of the media and increased NO$_3^-N$ availability to muskmelon seedlings. The increased root length and germination rate of muskmelon in the sanitized media suggest that these attributes determine plant survival.

Physical properties of the media are generally influenced by particle sizes and AFP of the composts, which thus contribute to plant growth and development. In the present study, suitable particle sizes were selected for the establishment of 10% AFP. These particles helped in holding moisture as small particles hold more water than the large particles. Moisture retention thus enhanced the germination and sustained the pH and EC in acceptable ranges. Physical properties of the media contributed a lot in enhancing germination of tomato and may affect the plant growth. This is well supported by the findings of who reported a differential convection of heat at various saturation and moisture levels. Penetration of steam through media is easier and higher in
Figure 3. Inorganic-N concentrations: NO$_3^-$–N and NH$_4^+$–N concentrations in muskmelon transplants as influenced by sanitation techniques. All the values are means ± S.E of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level.

Figure 4. Relative concentrations of nutrients in the media and muskmelon seedlings under different sanitation techniques. Concentrations of various nutrients: (a) total N; (b) P; (c) K in the media; (d) N; (e) P and (f) K in muskmelons transplants have been represented. All the values are means ± S.E of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level.
comparison to fumigants or dry heating. Additionally, microbial communities are affected by physical properties of the media. In the present study, increased WHC by the composts helped in the promotion of plant growth and development, whereas sanitation of the media influenced the population of nitrifying bacteria and inhibited pathogen attack on muskmelon. In general, nitrifying bacteria contribute significantly to N transformation and nitrification. Soil sterilization resulted in re-colonization of healthier microorganisms in the rhizosphere. Recurrent drying and wetting largely affects the microbial biomass. Similarly, warming of temperate forest soil altered the microbial community functioning.

The reduction in nitrifying bacterial community under steaming inhibited the nitrification potential of the media at 0 and 10 d intervals and maintained optimal NO$_3^-$:NH$_4^+$ ratios in muskmelon transplants. Since plant nutrient requirements, especially of N are very low at seedling stage, muskmelon growth in the present study was not affected rather synchronized with the needs of N of transplants at early stages. The increase in NO$_3^-$ at 20 and 30 d of sanitation were resulted due to recovery of nitrifying bacteria, which thus enhanced the nitrification of NH$_4^+$ to NO$_3^-$ and improved the growth of muskmelon seedlings. This implies that crop NO$_3^-$ requirements were high than NH$_4^+$-N. Previous studies exhibited that NH$_4^+$-N inhibited the maize growth, whereas the elevated levels of NO$_3^-$ improved the growth of maize. Therefore, suitable ratios of both N-forms are necessary for the optimum plant growth, otherwise NH$_4^+$-N cause toxicity in the plants. In the present study, NO$_3^-$:NH$_4^+$-N ratio was closely adjusted to optimal (2:1) during crop growth cycle to reveal the effect of steaming and formalin fumigation. However, best plants growth was observed at 75:25 ratio of NO$_3^-$ to NH$_4^+$-N.$^{38}$ These differences possibly resulted due to variations in crop genotypes, growth conditions, and growth medium.

The increase in nutrient uptake by muskmelon seedlings in sanitized media was resulted due to mineralization potential and WHC of the media. Since root lengths of muskmelon seedlings were increased in sanitized media, these interacted with the mineralized nutrient pool and absorbed water. Higher acquisition of K by muskmelon seedlings was achieved possibly due to competition between K and NH$_4^+$-N at root interface. This has been reported that K uptake was increased with the increase in NH$_4^+$-N.$^{38}$ In view of competition mechanism, anion like NO$_3^-$ favored in PO$_4^{3-}$ accumulation in muskmelon seedlings. In the present study, suitable NO$_3^-$:NH$_4^+$-N ratio synergistically contributed to the uptake of P. In another study, suitable NO$_3^-$:NH$_4^+$-N ratio enhanced P uptake by maize.$^{39}$ In addition, N:P ratios influence the fungi, bacterial colonization, availability and uptake of nutrients.$^{39}$

In spite of this, transplant shock under field conditions is obvious. Preventing transplants from shock, pathogenic attack and soil borne disease are major challenges in recent years. In the present study, media acted as biofumigants which reduced the widely distributed plants diseases like Fusarium wilt and pathogens like nematodes. Moreover, steaming of the media eradicated root nematode disease and Fusarium wilt more than formalin fumigation possibly due to increased NO$_3^-$ uptake which in turn provided resistance against pathogenic attack and transplant shock. Several media alternatives like vinegar residue and spent coffee increased the resistance in plants against Fusarium wilt$^{40}$ and enhanced the growth of basil and tomato.$^{41}$ In the present study, sanitation-induced

![Figure 5](https://doi.org/10.1038/s41598-021-99692-0)
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| Site | Address                  | Latitudes longitudes | Depth (cm) | Texture          | pH      | EC (dS m⁻¹) | SAR    | SOM (%) | N (%) | P (mg kg⁻¹ soil) | K (mg kg⁻¹ soil) |
|------|--------------------------|----------------------|------------|------------------|---------|-------------|--------|---------|-------|-----------------|-----------------|
| Site 1 | Ghallu, Mailsi            | 29.89° N 72.08° E    | 15         | Sandy loam       | 8.07 ± 0.32 | 0.86 ± 0.02 | 3.56 ± 0.12 | 0.76 ± 0.03 | 0.04 ± 0.002 | 6.41 ± 0.23 | 83 ± 3.92      |
| Site 2 | Lakhokha, Mailsi          | 29.88° N 72.06° E    | 15         | Loam             | 8.23 ± 0.29 | 1.03 ± 0.03 | 2.84 ± 0.11 | 0.91 ± 0.04 | 0.05 ± 0.002 | 7.93 ± 0.33 | 167 ± 7.42     |
| Site 3 | Marri Mitru, Mailsi       | 29.80° N 72.17° E    | 15         | Clay loam        | 8.34 ± 0.18 | 0.97 ± 0.04 | 3.11 ± 0.13 | 0.87 ± 0.04 | 0.05 ± 0.002 | 7.17 ± 0.27 | 173 ± 8.01      |

Table 2. Soil physico-chemical properties of the experimental sites. All the values means ± S.E of three replicates.

Effects on transplants growth persisted after transplantation and improved the growth of transplants under field conditions. The post-transplantation improvement in growth rate and yield of muskmelons was subjected to beneficial interaction effect of sanitation with guar and jantar and wheat straw media. Microbiome changes in rhizosphere led to decrease in root knot nematodes. Fumigation with ammonium bicarbonate and organic fertilizer, suppressed the *Fusarium wilt* to 12% in watermelon and enhanced the yield. Treating of plug trays at 65 °C for 60 min manifested in the reduction of *phytophathora*. In the present study, steaming provided resistance in the muskmelon transplants against root knot nematodes and *Fusarium wilt*, thereby improved the growth and yield.

Conclusions

Steaming sanitation decreased the prevalence of nitrifying bacteria and inhibited nitrification in steaming thus improved the NO₃⁻/NH₄⁺ ratios, P and K nutritional balance both in the media and muskmelon transplants than formalin fumigation. Additionally, steaming reduced pathogens and diseases in plants thus improved muskmelon growth and yield more than formalin fumigation. Based on our findings, steaming being a non-chemical and environment-friendly approach is recommended for soilless media. Further, optimization of steaming for various composts to use as media for various crops needs to be investigated with steaming technique.

Material and methods

Experimentation, climatic conditions and determination of disease severity. Muskmelon (*Cucumis melo* L. cv. Melon) nursery was raised in plug trays which contained all the media either sanitized or not. There were different plug trays for each of the media: wheat straw, guar, jantar, and rice hull and sanitation techniques: steaming and formalin. One seed in each hole of the plug trays was sown for 30 d. Germination percentage, mortality, seedling height, root length, number of true leaves per seedling and seedlings fresh weights were recorded.

Afterwards, seedlings were transplanted in three different fields located in Tehsil Mailsi, District Vehari, Punjab, Pakistan. Before transplantation, surface soil samples (0–15 cm depth) were collected for physico-chemical analysis (Table 2). Soils were prepared by conventional tillage practices and chemical fertilizers i.e. NPK were applied once at the time sowing from their sources: urea, di-ammonium phosphate (DAP), and sulphate of potash (SOP) at the rate of 200 kg N, 150 kg P₂O₅, and 110 kg K₂O ha⁻¹, respectively. The dimensions of the beds and furrows were: 150 cm wide × 60 cm wide, whereas 14,680 transplants ha⁻¹ were maintained. All the cultural and management practices were implemented throughout the experiment.

Plants received natural sunlight and other climatic conditions of the study area are: mean day/night temperature 32 °C/24 °C with 13 h photoperiod and 51–52% relative humidity.

Muskmelon was grown in the fields till maturity or 105 d, whereas symptomatic plants were randomly selected for the evaluation of nematode disease severity and *Fusarium wilt* during whole experiment. Muskmelon yield plant⁻¹ was measured at harvesting.

Preparation of composts and establishing required AFPs and sanitation treatments. Crop residues like wheat straw, jantar, guar and rice hull were subjected to composting by pit method, whereas peat moss (Peltracom N.V., Belgium) was used as a reference material. Particles of sizes > 5 mm, 3.3–5 mm, 2–3.3 mm, < 2 mm, 2–1 mm, 1–0.5 mm and < 0.5 mm were separated passing through sieves of various sizes viz. 0.5, 1, 2, 3, 3.3, and 5.0 mm. The mixes (substrates) were sequentially prepared with different AFPs (Tables S1 and S2). The required AFPs of the substrates were determined employing CEN standard. The substrates of 10% AFPs and peat moss were used as controls and were sanitized with formalin at the rate of 2 ml l⁻¹ or steaming at 60 °C for 30 min.

Determination of physic-chemical properties and fiber contents of the composts. Physico-chemical properties of the potting media like water holding capacity (WHC), bulk density and shrinkage percentage were determined (Table S3). Fiber contents of the substrates were separated by manual shaking in a container and their volume was quantified.

Determination root knot nematode sp. *melonoidyge* and *Fusarium wilt* sp. *melonis* population. Number of second stage juveniles (J2s) of root knot nematodes (*Meloidogyne* sp.) were determined using hemocytometer and expressed as number of juveniles (J2s) root knot nematodes kg⁻¹ of the media.
Determination of nitrifying bacteria in the composts. Composts samples were collected and brought to the laboratory under sterile conditions after 0, 10, 20 and 30 d (harvest) and abundance of nitrifying bacteria (CFUs) were determined by plating in the medium having chemical composition for all nitrifying bacteria. Briefly, 1 g of the media was mixed with 30 ml using sterile water and serial dilutions of 10\(^{-1}\) to 10\(^{-5}\) suspensions were spread on agar media.

Determination of nutrient concentrations in the composts and muskmelon seedlings. For the determination of nutrient concentrations, extracts from the media were collected by shaking 1:5 (w/v) at a speed of 150 rpm for 30 min and used for the measurements of NPK by Kjeldahl apparatus, spectrophotometer and flame photometer, respectively. For the purpose of NO\(^{-3}\)-N and NH\(^{+4}\)-N concentrations, composts mixes were extracted in 2 M KCl for one hour at a speed of 150 rpm and collected aliquots were used to determine NO\(^{-3}\)-N and NH\(^{+4}\)-N by steam distillation. Likewise, nutrients concentrations like total N, NO\(^{-3}\)-N, NH\(^{+4}\)-N, P, and K in muskmelon transplants were measured after digesting plant materials in acid digestion mixture HNO\(_3\):HClO\(_4\) (4:1 v/v).

Experimental design and statistical analyses. The experiment followed split plot design. In general, there were three replications in each treatment. One-way ANOVA was obtained for statistical evaluation of AFPs of different mixes, whereas two-way ANOVA was used for the interpretation of second stage juveniles (J2s) of disease-causing agents and mortality rate and muskmelon using Statistix 8.1 software. Treatment means of the yield data were compared according to Tukey’s post-hoc test. Generalized ANOVA was performed using SAS PROC Generalized Mixed Model (GLIMMIX) for analyses of the seedlings data. Since the data of variable germination was in the units of percent, modelling was employed by specifying the beta-binomial distribution (DIST = BETA). Data of root length, seedlings height, stem diameter, leaf area, and fresh weight variables were checked for normal distribution (DIST = LOGN). For these variables, standard error was merely usable on the natural log scale, hence estimates of the means are described with 95% confidence limits. Based on the fit statistics, (shifted) t distribution was found as suitable for model of the data of number of true leaves per seedling. Moreover, germination% and other seedlings growth attributes were modeled using class variables: compost type, sanitation type, and their interaction (compost x sanitation). Scheffe’s adjustments were made for multiple comparisons among the treatments.

Ethics approval and consent to participate. We all declare that manuscripts reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Complies with international, national and/or institutional guidelines. Experimental research and field studies on plants (either cultivated or wild), comply with relevant institutional, national, and international guidelines and legislation.
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**Author contributions**

Conceptualization, G.M., M.A.A. and S.M. methodology, G.M. M.A., M.F.Q. and A.U.R. software, T.S., A.H.K., S.M.; formal analysis, G.M., M.A.A., D.S., T.S., S.M.; investigation, G.M. and S.M.; resources, M.A.A., G.M., N.A. and M.F.Q.; data curation, G.M. and M.A.A.; writing—original draft preparation, G.M., S.M., M.A.K., S.F., R.D. and S.A.; writing—review and editing, S.M., M.A.A., G.M., S.F., R.D. and M.I. visualization, S.M. and M.A.A.; supervision, M.A.A. project administration, M.A.A. funding acquisition, M.A.A. All the authors have read and approved the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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