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**RESEARCH ARTICLE**

# EVALUATION OF PHYTOTOXICITY OF COMPOSTS PRODUCED FROM SPENT MUSHROOM SUBSTRATE USING SEED GERMINATION BIOASSAY OF *Raphunus sativus* L.

Amarasinghe SR[1]* and Jayaweera WMCS[2]

[1]Department of Soil Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, 81100, Sri Lanka.

[2]Department of Biosystem Technology, Faculty of Technology, University of Ruhuna, Kamburupitiya, 81100, Sri Lanka.

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**ABSTRACT**

**Spent mushroom substrate (SMS) has the potential to produce compost with different additives. However, some substances and by-products produced during the process can be toxic to seed germination and plant growth. Evaluation of the phytotoxicity is important for guaranteeing compost quality. Therefore, this study was conducted to evaluate the phytotoxicity of spent mushroom substrate compost (SMSC) using a seed germination bioassay. Compost aqueous extracts (CAEs) were prepared from SMSC produced by amending different additives (green leaves and urea) with and without an inoculum. Dilution series of 25%, 50%, and 100% were tested against seed germination of radish (*Raphanus sativus* L). Germination index (GI) in all 25% CEAs of this study were phytotoxic free and they indicated the availability of phytonutrients and/or phyto-stimulants except the non-inoculated SMSC without amendments. Similarly, all 50% CAEs were phytotoxic free except *Tithonia* amended inoculated SMSC with less phytotoxicity. Of 100% CAEs, non-inoculated *Pueraria* amended SMSC was highly phytotoxic, non-inoculated *Tithonia* and urea amended SMSC were phytotoxic free, and the rest were less phytotoxic. Seed germination of *R. sativus* was not inhibited by any of the CAEs of *Tithonia* amended SMSC indicating the higher usability as an amendment in SMS. The water extractable $NH_4^+$ concentration was significantly higher ($p<0.05$) in the inoculated SMSC amended with urea and it was significantly lower ($p<0.05$) in the non-inoculated SMSC amended with *Tithonia* and inoculated SMSC without amendments. Further, the inoculated SMSC extracts showed higher performance in GI than non-inoculated SMSC. GI showed a higher negative significant correlation ($r=-0.841$, $p<0.01$; $r=-0.778$, $p<0.01$) with EC in both inoculated and non-inoculated compost samples, respectively. Moreover, the EC was significantly correlated with all extractable tested ions except $Cu^{2+}$ and $Ni^{2+}$ in inoculated treatments. pH values in all aqueous extracts were recorded in the range of 6.27 to 8.08. Extractable elements $Cu^{2+}$ ($r=0.645$, $p=0.01$) and $Zn^{2+}$ ($r=0.577$, $p=0.05$) had positive correlation with pH. SMS can be recycled as compost integrating nitrogenous additives and inoculum to use safely as a soil amendment.**

Keywords: Compost, Phytotoxicity, Seed germination, Spent mushroom substrate

## INTRODUCTION

Edible mushroom cultivation is popular worldwide due to its fast-growing nature and the possibility to obtain higher yields within a short period. The continuous yield can be obtained for about three months. Afterwards, the spent mushroom substrate (SMS) has no longer usable among farmers and piles up or burns into ash.

Therefore, SMS is considered as waste that is obtained from the mushroom production industry. Piling up SMS after mushroom harvesting may cause various environmental problems including water contamination and nuisance (Beyer 1996). This could be re-

*Corresponding author: rajika@soil.ruh.ac.lk

utilized to produce mushrooms or used as a direct soil amendment. The growth of unwanted microorganisms in compost produced from SMS is one of the problems in utilizing it as a soil amendment. However, the SMS can be directly used as "fresh mushroom compost" after being pasteurized under steam heat (Davis and Fidanza 2011) or by the hot composting process for at least 18 days to inactivate the unwanted fungi and weed seeds (Peters *et al*. 2000). Then the secondary pile can be formed to complete the composting process up to 90 days or less depending on the substrate. In Sri Lanka, the SMS is mostly piled up on the ground. However, these are not abundantly re-utilized in Sri Lanka as compost or any other utility.

SMS can be used to prepare compost which is used as an organic amendment. It is within the perfect range for compost production having 35% to 55% (wet weight) solids and 45% to 65% (wet weight) moisture content (Stoffella and Kahn 2001).

Compost should be free of salinity, $NH_4^+$-N, heavy metals, etc. by metabolization or immobilization at maturity ensuring free phototoxicity (Emino and Warman, 2004). Major constraints of the SMS would be the salinity which is concentrated by added lime ($CaCO_3$ and $MgSO_4.7H_2O$) in mushroom production. Therefore, a risk remains due to a high salinity problem in the final spent mushroom substrate compost (SMSC). Besides salts, SMS contain $NH_4^+$-N, heavy metals and metal pollutants, such as arsenic, chromium, nickel, and selenium depending on the initial substrate (Alvarenga *et al.* 2015) which may create phytotoxicity (Wollan *et al.* 1978; Wong *et al.* 1983). High salinity levels may affect germinating seeds and young seedlings (Kebrom *et al.* 2019) and are harmful to plant growth (Singh and Kalamdhad 2013a; 2013b). The damage may be severe to salt-sensitive plants.

The bioremediation by microorganisms can eradicate some toxicants by biological mechanisms to innocuous forms (Ojuederie and Babalola 2017). The availability of these toxicants is reduced by adjusting pH, the redox reactions and the adsorption of pollutants (Jain and Arnepalli 2018). Redox reactions can be operated in the composting process (Bolan 2013). It helps to increase microbial population by altering pH, decreasing the heavy metals, and increasing allochthonous microbial biomass and plant nutrients (Alburquerque *et al.* 2011; Chen *et al.* 2015) Microorganisms utilize different processes such as precipitation, biosorption, enzymatic conversions of heavy metals (Ojuederie and Babalola 2017). Introducing microorganisms by inoculation may help in such processes.

Phytotoxicity assessment is mandatory as a parameter in the quality profiling of compost, before utilizing them. Even though different types of chemical analysis have been carried out by many researchers (Marambe *et al.* 1992; Ramírez *et al.* 2008; Hase and Kawamura 2012; Fuentes *et al.* 2004; Tang *et al.* 2008; Siles-Castellano *et al.* 2020) to identify potential toxicants such as organic products, $NH_4^+$-N, heavy metals, pesticide residues, many are impractical due to time intensity and expansiveness. Further, the interactive effects of these toxicants cannot be evaluated properly through chemical analysis.

Therefore, calculating the germination index (GI) is a reliable method to ascertain the phytotoxic effect of compost and it evaluates the phytotoxins which can temporarily or permanently affect the growth of a plant (Warman 1999). Thus, seed germination-related bioassays have been conducted as an effective, low cost and rapid method to determine phytotoxicity (Mazumder *et al.* 2020). Such seed germination experiments can be conducted using aqueous extracts of organic amendments and incubating the selected seed varieties (Emino and Warman 2004; Barral and Paradelo 2011). Many previous studies have followed seed germination protocols as a bioassay (Kebrom *et al.* 2019). The seed germination index has introduced by Zucconi *et al.* (1981) for cress seeds (*Lepidium sativum*, L.) by considering seed radicle length and germination percentage by comparing the control in deionized water. Later Gariglio *et al.* (2002) utilized lettuce, whereas Emino and Warman

(2004) used 14 small to large-sized seed species such as Amaranthus (*Amaranthus tricolor*), bean (*Phaseolus vulgaris*), carrot (*Daucus carota*), radish (*Raphanus sativus*), etc. Many researchers have evaluated GI using *Raphanus sativus* seeds which are sensitive to toxicants at the early germination cycle (<48 h) and the convenient medium-seed size (Iannotti *et al.* 1994; Gajdos 1997; Luo *et al.* 2018).

As the first step of the seed germination test, the compost aqueous extracts should be prepared according to the suitable extraction ratio. There is no universal ratio of extraction and many researchers have adopted several extraction ratios (Luo *et al.* 2018). Some researchers have implemented 1:10 (w/v) aqueous extracts (Guo *et al.* 2012; Yang *et al.* 2013; Huang *et al.* 2016; Luo *et al.* 2018. Also, Said-pullicino *et al.* (2007), Pampuro *et al.* (2010) and Cesaro *et al.* (2015) have standardized the moisture content for 85% by adding deionized water into the fresh sample after determining the dry matter content of each sample. Hence, many studies have used different extraction methods, seed types and numbers, the volume of the extract and incubation time for the test. Of these, the majority have utilized Petri dishes to test the radicle elongation (Luo *et al.* 2018).

In this study, we assumed that SMS integrated with other amendments will affect the seed germination and root elongation due to the phytotoxic nutrient elements, heavy metals and metal pollutants present and the added inoculum may accelerate the decomposition and reduce the phytotoxicity. As phytotoxicity assessment is a significant and compulsory parameter to determine the quality of SMSC, the seed germination bioassay of radish (*Raphanus sativus* L.) seeds using aqueous extracts of each final compost mixtures were tested to evaluate the possible phytotoxic effect of these compost mixtures.

## MATERIALS AND METHODS

### Spent mushroom substrate for compost

SMS of oyster mushroom (*Pleurotus ostreatus*) obtained from mushroom production unit of Faculty of Agriculture, University of Ruhuna, Sri Lanka was used as the key waste to prepare compost. Green leaves i.e. *Peuraria pasioloids* leaves, *Gliricidea sepium* leaves, *Tithonia diversifolia* (wild sunflower) leaves, and inorganic nitrogen fertilizer (urea) were used as N sources. The total C%, total N% content and moisture content were analyzed in all the raw materials. The SMS was amended and mixed thoroughly with the green leaves and urea separately according to the amount of materials calculated using formula 1 (Rynk *et al.* 1992). The C/N ratio of the final compost mixture for optimum decomposition was considered 25:1.

$$\mathbf{R=[Q_1C_1(100-M_1)+Q_2C_2(100-M_2)] \ / \ [Q_1N_1(100-M_1)+Q_2N_2(100-M_2)]} \quad \text{..... Eqn 1}$$

where R is the C/N ratio of final compost mixture for optimum decomposition, $Q_1$ is the known weight of sample (A), $C_1$ is the known C% of sample (A), $M_1$ is the known moisture% of sample (A), $M_2$ is the known moisture % of sample (B), $N_1$ is the known N% of sample (A), $Q_2$ is the unknown weight of sample (B) to be added, $C_2$ is the known C% of the sample (B) and $N_2$ is the known N% of sample (B).

Then, another five mixtures were prepared similarly and inoculated with an inoculum/ biodynamic formulation (Amarasinghe and Gunawardhana 2020). Two treatments were prepared with SMS without amending green leaves or urea but mixing with inoculum and without inoculum, respectively. Each compost mixture of 2 kg was separately put into a black polythene bag of gauge 150 and labelled accordingly. All the treatments were replicated three times and allowed to decompose aerobically. The bags were arranged in ambient temperature and humidity conditions using a Complete Randomized design (CRD). Every compost mixture was mixed thoroughly every other day to speed the decomposition process. Moisture content was adjusted by applying water by monitoring the moisture level by field method. Mixtures were allowed to decompose for 90 days.

**Preparation of inoculum**

To prepare the inoculum, 500 g of cow dung, 250 ml of cattle urine, 100 g of brown sugar, 100 g of mung bean flour and one handful of topsoil from an undisturbed forest area were collected and added to a 10 L bucket. The contents were mixed well and increased volume up to 10 L by adding water. The mixture was stirred well to aerate once in two days and kept for 10 days. The inoculum was applied at a rate of 500 L/100 kg of substrate considering the bulk density of the substrate. The bulk density of coarse sawdust was 340 kg /$m^3$.

**Determination of Germination index (GI)**

In the present study, a germination bioassay was performed on aqueous extracts of mature SMSC using *Raphanus sativus* seeds. *R. sativus* seeds were used due to the sensitivity to toxicants in the early germination cycle and convenient seed size. Certified *R. sativus* seeds with 75% germination were used to determine the GI. GI test was conducted according to Kebrom *et al.* (2019). CAEs from ten mature SMSC, (i) SMS + *Gliricidea sepium* leaves (ii) SMS + *Gliricidea sepium* leaves with inoculum (iii) SMS + *Tithonia diversifolia* leaves (iv) SMS + *Tithonia diversifolia* leaves with inoculum (v) SMS + *Peuraria pasioloids* leaves (vi) SMS + *Peuraria pasioloids* leaves with inoculum (vii) SMS + urea (viii) SMS + urea with inoculum (ix) SMSC without inoculum (x) SMSC with inoculum were used for the seed germination bioassay.

**Sample preparation and physicochemical characterization**

Matured compost mixtures were dried overnight at 105 $^oC$ in an oven. The dry matter and the moisture content were determined. Then it was standardized up to 85% moisture content by adding deionized water. By Shaking the samples on a horizontal shaker in a volumetric flask for two hours and solutions were filtered using a Whatman no. 01 filter paper. Three concentrations (25%, 50%, and 100%) of the aqueous extracts were prepared to assure the accuracy of the evaluation. pH, electrical conductivity (EC), water-extractable $NH_4^+$, $Fe^{3+}$ $Cu^{2+}$, $Ni^{2+}$, $Cr^{3+}$, $Zn^{2+}$, $Na^+$, $SO_4^{2-}$, and $Ca^{2+}$ concentrations of each aqueous extracts were analyzed. pH and EC were measured using a pH and EC meter (HANNA HI 83099). Flame atomic emission spectrometry was used to analyze $Ca^{2+}$ and $Na^+$ (Tandon 1995). $SO_4^{2-}$, $NH_4^+$ and heavy metals were analyzed using a photometer according to the colourimetric methods (CHEMetrics 2018). The seeds were dipped in a 10% calcium hypochlorite solution for surface sterilization (Kim *et al* 2011) for 10 mins and the seeds were rinsed with sterilized water. Five radish seeds were placed on a Petri dish (9 cm) with lined qualitative filter paper. Three replicated dishes were prepared for each compost aqueous extract in different concentrations (25%, 50%, and 100%). Five millilitres of the extract were poured into each treatment Petri dish and 5 ml of deionized water to the control peri dish. The experiment was arranged in a complete randomized design. The GI test was conducted under the dark condition at room temperature (27 $^oC$). The lids were kept on the Petri dish to prevent evaporation. The number of seeds germinated following 72 h incubation was counted and the radicle of the seedlings were measured. According to the standard formulas, (Kebrom *et al.* 2019, Mazumder *et al.* 2020) percentages of RSG, RRG and GI were calculated (equations 2, 3 and 4).

**RSG = [Number of seeds germinated in compost aqueous extract / Number of seeds germinated in control] x 100% …… Eqn 2**

**RRG = [Mean radicle length of germinated seeds in compost aqueous extract (mm) / Mean radicle length of germinated seeds in control (mm)] x 100% …… Eqn 3**

**GI = [RSG x RRG] x 100% …… Eqn 4**

Where RSG is the relative seed germination, RRG is the relative root growth and GI is the Germination index.

The above three terms are relative terms which were compared to the control having always 100. Then, whenever values of treatment exceed the control in either germination or root elongation, the values will

exceed 100 and the GI may be higher than 100 (Emino and Warman, 2004).

**Statistical Analysis**

The number of germinated seeds and the average radicle length in each of the replicates were determined. The RSG, RRG, and GI of each were calculated. The results reported in this paper are the mean GI, RSG, and RRG of treatments, and the error bars are the standard error of the mean (SE). The results of physical and chemical properties were also analyzed similarly. Treatment means were tested by one-way ANOVA followed by a Duncan Multiple Range Test (DMRT) for the mean separation. A correlation analysis was conducted between GI, RSG, RRG and other physicochemical parameters of treatments. All analyses were performed using SPSS 17.0. (SPSS 2006).

## RESULTS AND DISCUSSION

**Germination of *Raphanus sativus* seeds in different CAEs (25%, 50%, and 100%) of different treatments and deionized water (control)**

GI is the most important parameter to evaluate the phytotoxicity of mature compost as it directly examines the effect of compost on seed germination and seedling growth (Wang *et al.* 2020). Table 1 shows the mean values of GI in 25%, 50%, and 100% CAEs. According to Emino and Warman (2004), compost with GI values ≤50% is highly phytotoxic, between 50% and 80% are less phytotoxic, ≥80% are phytotoxic free, and 0 indicates extreme phytotoxicity. The availability of phytonutrients and/or phyto-stimulants is the reason for GI higher than 100% (Emino and Warman 2004). Correspondently, all 25% CAEs of this study were phytotoxic free, and they indicated the availability of phytonutrients and/or phyto-stimulants except the non-inoculated SMSC without amendments. Similarly, all 50% CAEs were phytotoxic free except *Tithonia* amended inoculated SMSC with less phytotoxicity. Of 100% CAEs, non-inoculated *Tithonia* and urea amended SMSC were phytotoxic free, non-inoculated *Pueraria* amended SMSC was highly phytotoxic, and the rest were less phytotoxic. Emino and Warman (2004) found that 25% and 50% CAEs are not phytotoxic, presumably diluted to a non-phytotoxic concentration resulted in a GI>80%, while the dilution to 75% CAE and undiluted 100% immature CAEs resulted in a GI≤20% and are toxic which resemble the results of the present study.

**Table 1: Means of the GI values of different CAEs**

| Treatment | | GI (%) | | |
|---|---|---|---|---|
| | | **25% CAE** | **50% CAE** | **100% CAE** |
| **SMS+*Gliricidia*** | non-inoculated | 153 a* | 105 e | 68 cd |
| | inoculated | 120 h | 95 g | 67 d |
| **SMS+*Tithonia*** | non-inoculated | 102 i | 86 h | 87 b |
| | inoculated | 152 b | 73 i | 74 c |
| **SMS+*Pueraria*** | non-inoculated | 123 g | 94 g | 40 f |
| | inoculated | 135 e | 109 d | 72 c |
| **SMS+Urea** | non-inoculated | 127 f | 92 g | 82 b |
| | inoculated | 144 e | 125 b | 60 e |
| **non amended SMSC** | non-inoculated | 92 j | 111 c | 72 c |
| | inoculated | 140 d | 134 a | 57 e |
| **Control (Deionized water)** | | 100 i | 100 f | 100 a |

***** Mean values designated by the same letter are not significantly different at $p<0.05$ as determined by DMRT.

Means of the GI values of CAEs were compared using DMRT (Table 1) to see the significant differences among calculated GI values among treatments.

In most treatments of 25% and 50% CAEs, GI percentages were significantly ($p<0.05$) higher than the control except for the 100% CAE. Emino and Warman (2004) indicated that GI may be higher than the control, whenever the values of treatments exceed the control in either germination or root elongation. However, within different CAEs, there were significant differences ($p<0.05$) among different treatments (Table 1).

The significantly lowest GI value at 25% CAE was observed in the treatment of non-inoculated SMSC without amendments while the highest was in *Gliricidia* amended non-inoculated SMSC ($p<0.05$). The GI was increased as SMSC without amendments <*Tithonia* amended SMSC<*Pueraria* amended SMSC<urea amended SMSC<*Glyricidia* amended SMSC in non-inoculated compost, while *Glyricidia* amended SMSC<*Pueraria* amended SMSC<SMSC without amendments<Urea amended SMSC<*Tithonia* amended SMSC in inoculated compost.

The GI in 50% CAE, *Tithonia* amended SMSC was significantly lower ($p<0.05$) compared to all treatments while the highest ($p<0.05$) was observed in inoculated SMSC without amendments. The GI was increased as *Tithonia* amended SMSC<urea amended SMSC< *Pueraria* amended SMSC<*Gliricidia* amended SMSC<SMSC without amendments in non-inoculated compost, while *Tithonia* amended SMSC<*Gliricidia* amended SMSC<*Pueraria* amended SMSC<urea amended SMSC< SMSC without amendments in inoculated compost. In both 25% and 50% CAEs, the GI was higher than the control (100%) in most treatments showing the phyto-stimulant behaviour of these compost extracts resembling the study of Siles-Castellano *et al.* (2020).

The GI in 100% CAE, *Pueraria* amended SMSC was significantly lower ($p<0.05$) than all the other treatments and the control irrespectively to inoculated and non-inoculated composts. However, CAE of 100% showed significantly higher ($p<0.05$) GI values in non-inoculated *Tithonia* amended SMSC and urea amended SMSC compared to the other non-inoculated treatments. The GI of non-inoculated *Pueraria* amended SMSC (40%) indicated the presence of phytotoxic substances comparable to the study conducted by Kebrom et al. (2019) using chicken manure, milorganite, and dairy manure as three commercial organic amendments. The GI was increased as *Pueraria* amended SMSC< *Gliricidia* amended SMSC< SMSC without amendments< urea amended SMSC<*Tithonia* amended SMSC in non-inoculated compost, while SMSC without amendments<urea amended SMSC< *Gliricidia* amended SMSC< *Pueraria* amended SMSC< *Tithonia* amended SMSC in inoculated compost.

Radicle elongation is a sensitive measure contributing to the GI (Emino and Warman 2004). To obtain the effect of inoculum in seed radicle elongation, it is important to find a relationship between GI and mean radicle length. Figure 1 shows the relationship between GI and means radicle length of *R. sativus* seeds in inoculated and non-inoculated treatments. Accordingly, the GI showed a positive linear relationship with the mean radicle length of *R. sativus* seeds. Further, it was clear that the inoculated treatments had a higher positive relationship ($r^2=0.9235$) with GI, compared to the non-inoculated treatments ($r^2=0.722$). Beneficial microorganisms may enhance the bioremediation process by reducing heavy metals and organic pollutants (Ojuederie and Babalola 2017). According to him, microorganism consortia in biofilms can be utilized to remediate heavy metals based on cell metabolism. Further, Sobarzo-Bernal *et al.* (2021) observed an enhanced radicle length by a biostimulant, which indicated the effect of microbial activity on radicle elongation.

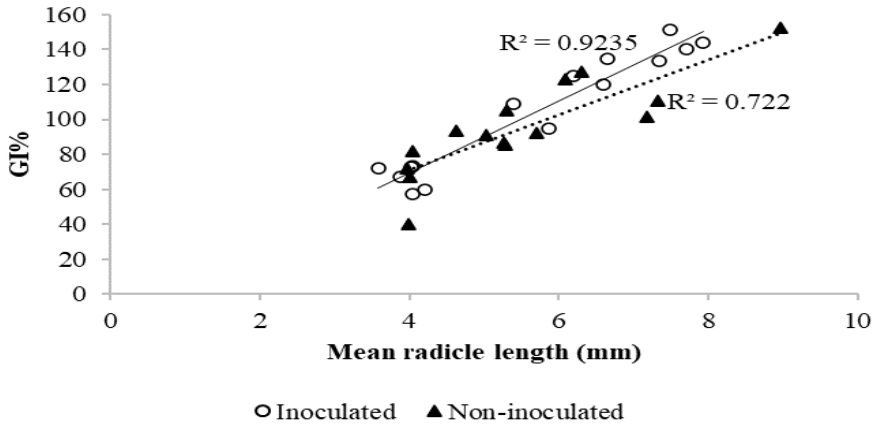


**Figure 1: Relationship between GI and mean radicle length of *R. sativus* seeds in inoculated and non-inoculated treatments**

**pH, EC and some elemental concentrations in different CAEs (25%, 50%, and 100%) of different treatments and deionized water (control)**

pH and EC values are important in seed germination. Figure 2 shows the relationship between GI and mean EC of CAEs in inoculated and non-inoculated treatments. Accordingly, there is a linear negative relationship with the mean EC for the inoculated SMSC ($r^2$=0.7064) and for non-inoculated SMSC ($r^2$=0.6009). It is visible that in low EC concentrations, the GI in CAEs of inoculated SMSC is higher than the non-inoculated SMSC.

In the present study, pH values for all extracts were in the range of 6.27 to 8.08 (data not shown), similar to the range of typical compost (Cayuela *et al.* 2008). According to Barral and Paradelo (2011), higher pH and EC values in compost inhibit seed germination. However, salinity effects are generally negligible when the EC value is ≤2000 µS/cm (Hoekstra *et al.* 2002). Alkaline pH values (>9) obtained in a seed germination study conducted by Siles-Castellano *et al.* (2020) have explained the low GI values, lower than 55%.

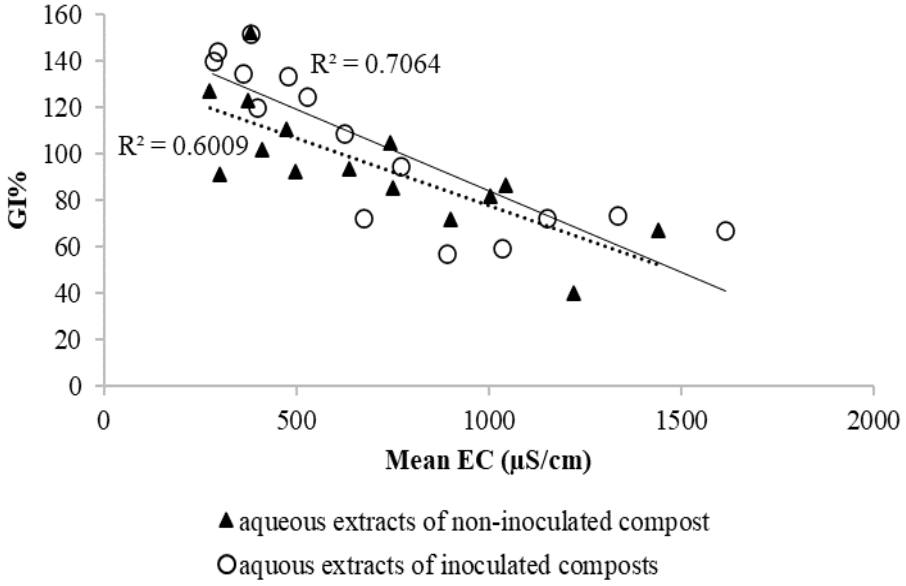


**Figure 2: Relationship with GI and mean EC of compost aqueous extracts in inoculated and non-inoculated treatments**

**Chemical properties and compositions of CAEs**

Chemical analysis of the CAEs is needed to identify potentially phytotoxic compounds. In the present study, phytotoxic analysis was performed only for the 100% CAE, since higher germination inhibition was observed in it. Figure 3 depicts the $Na^{+}$ and $Ca^{2+}$ composition in the compost aqueous extracts.

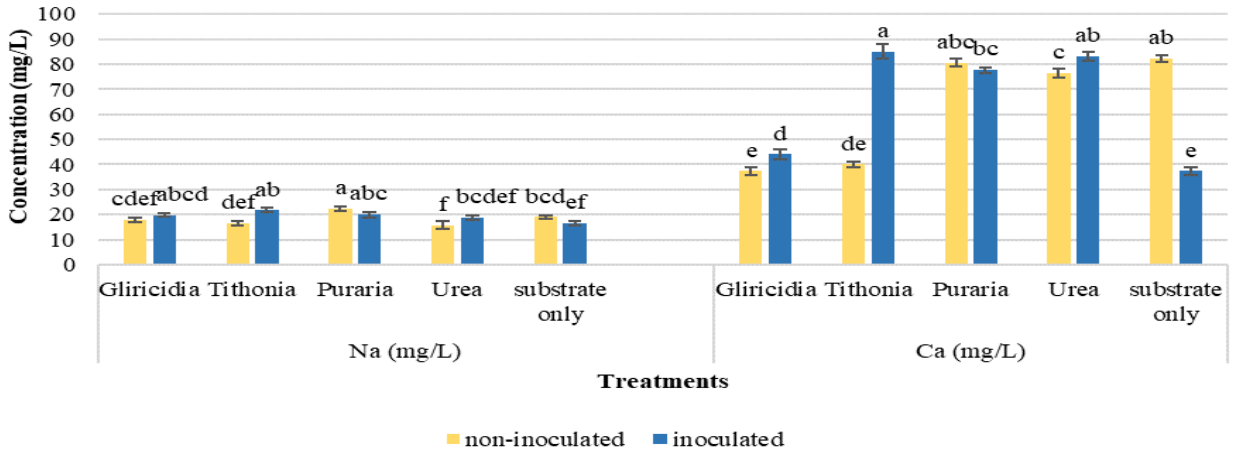


**Figure 3: $Na^{+}$ and $Ca^{2+}$ composition in the 100% CAE** (Vertical bars represent standard error (mean ± SE) and mean values designated by the same letter are not significantly different at $p<0.05$ as determined by DMRT)

The average $Na^{+}$ content was significantly lower ($p<0.05$) in non-inoculated urea amended SMSC compared to other treatments except for non-inoculated *Gliricidia* amended SMSC, non-inoculated *Tithonia* amended SMSC and inoculated SMSC without amendment. Kebrom *et al.* (2019) have found that the level of $Na^{+}$ in the aqueous extracts was related to their EC values which were similar to the present study (Tables 2 and 3).

The average $Ca^{2+}$ content was significantly lower ($p<0.05$) in non-inoculated *Gliricidia* amended SMSC, non-inoculated *Tithonia* amended SMSC and inoculated SMSC without amendment compared to other treatments. A higher $Ca^{2+}$ concentration may obtain due to the availability of $CaCO_3$ in the SMS.

Figure 4 depicts the $Cu^{2+}$, $Fe^{2+}$ and $Zn^{2+}$ composition in the 100% CAE.

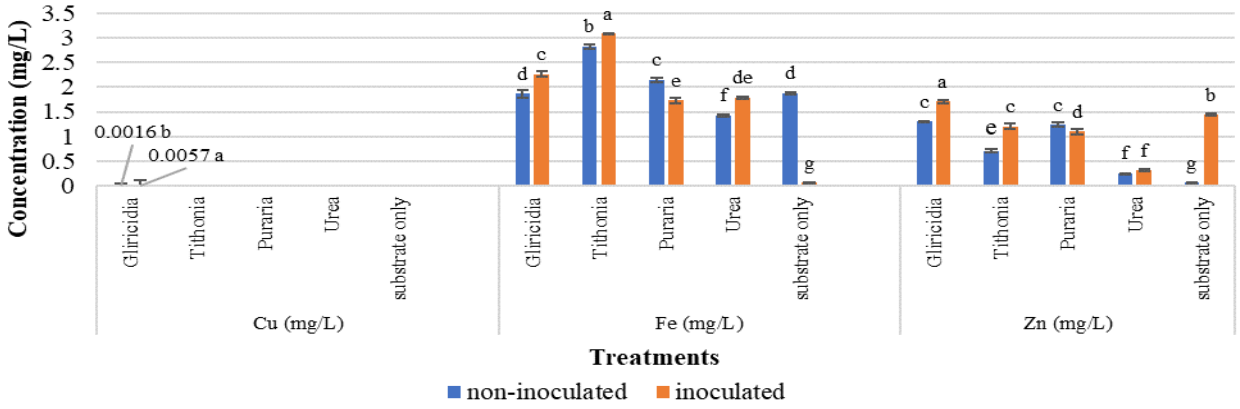


**Figure 4: $Cu^{2+}$, $Fe^{2+}$ and $Zn^{2+}$ composition in the 100% CAE** (Vertical bars represent standard error (mean ± SE) and mean values designated by the same letter are not significantly different at $p<0.05$ as determined by DMRT)

Metals significantly inhibit germination and plant growth (Wollan *et al.* 1978). Further, the heavy metal concentration in compost is considered a restrictive factor which is essential to analyze for its safe application as an organic amendment to the soil (Alvarenga *et al.* 2015). Plant micronutrients such as zinc ($Zn^{2+}$), copper ($Cu^{2+}$), and iron ($Fe^{3+}$) had significant differences in treatments in this study. $Cu^{2+}$ was detected only in *Gliricidia* amended SMSC. It was significantly different ($p<0.05$) between inoculated and non-inoculated while inoculated was higher. The amount of $Fe^{3+}$ was significantly different in most of the treatments. It was significantly higher ($p<0.05$) in inoculated *Tithonia* amended SMSC followed by its non-inoculated treatment than other treatments. $Fe^{3+}$ content was significantly lower ($p<0.05$) in inoculated SMSC without amendments followed by non-inoculated urea amended SMSC. Water extractable $Cu^{2+}$ concentrations in the cattle manure valuing 0.04 mg/L inhibited the root growth of the cress.

In most of the treatments, $Zn^{2+}$ content was significantly different compared to each other. $Zn^{2+}$content was significantly higher ($p<0.05$) in inoculated *Gliricidia* amended SMSC followed by inoculated SMSC without amendments. Significantly lower ($p<0.05$) $Zn^{2+}$ content was recorded at non-inoculated SMSC without amendments. According to Davies (1977) $Zn^{2+}$ contents of SMSC have not exceeded the critical range of 75 to 600 mg/L.

Figure 5 shows the $Cr^{2+}$ and $Ni^{2+}$ composition in the 100% CAE. Potentially toxic heavy metals including chromium ($Cr^{2+}$), and nickel ($Ni^{2+}$) were observed in most of the treatments of this study.

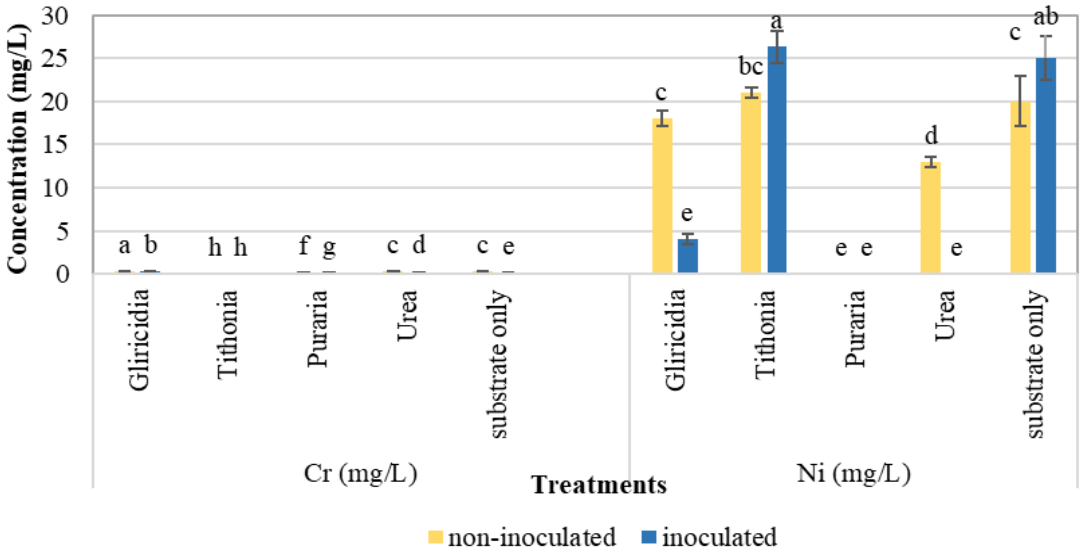


**Figure 5: $Cr^{2+}$ and $Ni^{2+}$ composition in the 100% CAE** (Vertical bars represent standard error (mean ± SE) and mean values designated by the same letter are not significantly different at $p<0.05$ as determined by DMRT)

However, $Cr^{2+}$ content was comparatively low. Among the treatments $Cr^{2+}$ content was significantly higher ($p<0.05$) in non-inoculated *Gliricidia* amended SMSC and lower ($p<0.05$) in *Tithonia* amended SMSC. $Ni^{2+}$ content was significantly higher ($p<0.05$) in inoculated *Tithonia* amended SMSC compared to other treatments except inoculated SMSC without amendment. $Ni^{2+}$ was not detected in *Pueraria* amended SMSC and inoculated urea amended SMSC. Kebrom *et al.* (2019) suggest that the phytotoxic effect in their respective study of them could be due to $Ni^{2+}$.

Figure 6 depicts the $SO_4^{2-}$ and $NH_4^+$-N composition in the 100% CAE. $NH_4^+$-N are possible compounds that generate

phytotoxicity in seed germination (Ramírez *et al*., 2008).

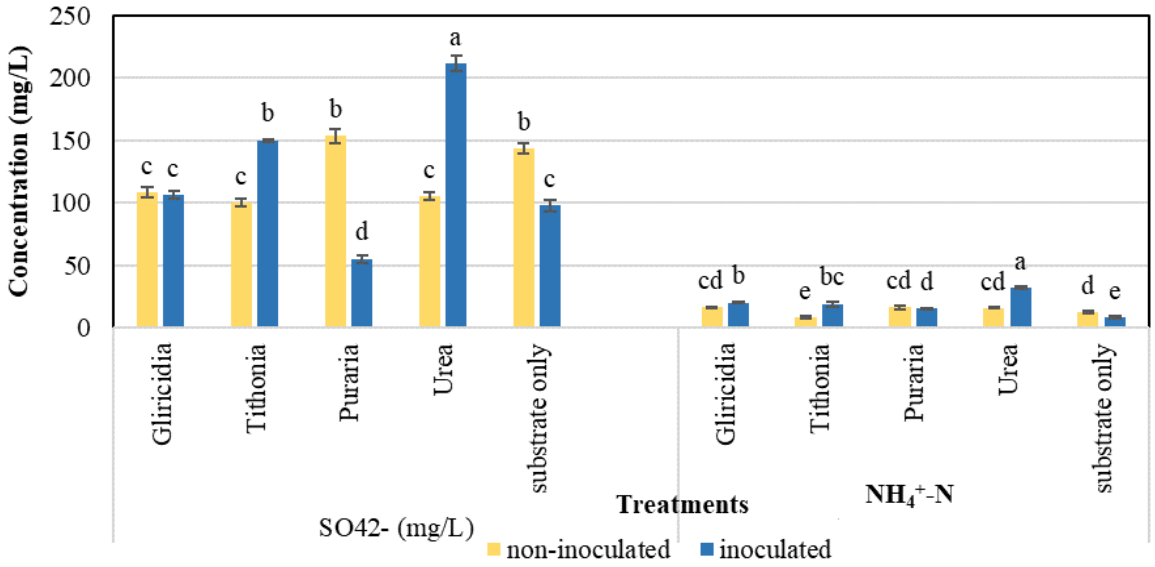


**Figure 6: $SO_4^{2-}$ and $NH_4^+$-N composition in the 100% CAE** (Vertical bars represent standard error (mean ± SE) and mean values designated by the same letter are not significantly different at $p<0.05$ as determined by DMRT)

Any of the inorganic forms of nitrogen (ammonia, nitrite and nitrate) in mature compost is of vast importance to determine its quality (Cesaro *et al.* 2019). In the present study, $NH_4^+$ content was significantly higher ($p<0.05$) in inoculated urea amended SMSC and significantly lower ($p<0.05$) in non-inoculated *Tithonia* amended SMSC and inoculated SMSC without amendments. The highest value of $NH_4^+$ concentration in urea may be due to the higher mineralization of amended urea which contains a higher amount of total N% (46%). According to Hoekstra *et al.* (2002), $NH_4^+$ of 633 mg/L has been demarcated as a phytotoxic level for compost extracts. However, none of the treatments was found to have this critical level in the present study.

There were no significant differences among most of the treatments in $SO_4^{2-}$ content. However, it was significantly higher ($p<0.05$) in inoculated urea amended SMSC while significantly lower ($p<0.05$) in inoculated *Pueraria* amended SMSC. The compost extracts showed a higher amount of $SO_4^{2-}$ may be due to added Epsom salt in the mushroom production substrate.

**Pearson correlation coefficients among various parameters of CAEs**

A correlation analysis was conducted between GI, RSG, RRG and other chemical parameters of inoculated and non-inoculated CAEs separately, to observe if any relationships between biological and chemical parameters (Tables 2 and 3). They probably indicate the indirect effects of phytotoxicity.

GI showed a higher negative significant correlation ($r=-0.841$, $p<0.01$; $r=-0.778$, $p<0.01$) with EC in both inoculated and non-inoculated compost samples, respectively. Pearson correlation of pH and Cu was found to be moderately positive and statistically significant ($r=0.645$, $p<0.01$) in inoculated treatments while pH and Zn showed a moderately positive and statistically significant ($r=0.577$, $p<0.05$) relationship in non-inoculated treatments. In the case of EC, there were positive significant correlations with Cr ($r=0.612$, $p<0.05$), Fe ($r=0.822$, $p<0.01$), Zn ($r=0.828$, $p<0.01$) in inoculated CAEs and with Cr ($r=0.625$, $p<0.05$), Fe ($r=0.861$, $p<0.01$), Zn ($r=0.785$, $p<0.01$), Ni ($r=0.517$, $p<0.05$) in non-inoculated CAEs (Table 3). When considering the correlation of

EC and other cations ($Na^{+}$, $Ca^{2+}$, $NH_4^{+}$) and anions ($SO_4^{-2-}$) a positive and significant correlation was observed in both inoculated and non-inoculated aqueous extracts.

On the other hand, in inoculated CAEs, Cr, Fe and Zn showed highly negative and statistically significant relationships (r=-0.555, p<0.05; r=-0.634, p<0.05; r=-0.713, p<0.01) with GI, respectively. Similar relationships were observed in Fe and Zn (r=-0.766, p<0.01; r=-0.544, p<0.05) in non-inoculated extracts.

RSG in inoculated CAEs showed a significant negative correlation with $SO_4^{2-}$ (r=-0.616, p<0.05) while RSG in non-inoculated extracts did not show a significant relationship with any of the parameters.

RRG in inoculated CAEs showed significant negative correlations with EC (r=-0.887, p<0.01), Cr (r=-0.521, p<0.05), Fe (r=-0.717, p<0.01), Zn (r=-0.738, p<0.01), Na (r=-0.924, p<0.01), Ca (r=-0.777, p<0.01), $SO_4^{2-}$ (r=-0.592, p<0.05), and $NH_4^{+}$ (r=-0.712, p<0.01). RRG in non-inoculated CAEs showed significant negative correlations with EC (r=-0.725, p<0.01), Fe (r=-0.734, p<0.01), Na (r=-0.789, p<0.01), Ca (r=-0.753, p<0.01), $SO_4^{2-}$ (r=-0.780, p<0.01), and $NH_4^{+}$ (r=-0.755, p<0.01).

Further, GI in inoculated CAEs showed significant negative correlations with Na (r=-0.889, p<0.01), Ca (r=-0.734, p<0.01), $SO_4^{2-}$ (r=-0.671, p<0.01), and $NH_4^{+}$ (r=-0.717, p<0.01). GI in non-inoculated CAEs showed significant negative correlations with Na (r=-0.808, p<0.01), Ca (r=-0.722, p<0.01), $SO_4^{2-}$ (r=-0.800, p<0.01), and $NH_4^{+}$ (r=-0.807, p<0.01).

EC in inoculated CAEs showed significant positive correlations with Na (r=0.947, p<0.01), Ca (r=0.738, p<0.01), $SO_4^{2-}$ (r=0.639, p<0.05), and $NH_4^{+}$ (r=0.779, p<0.01). EC in non-inoculated CAEs showed significant positive correlations with Na (r=0.910, p<0.01), Ca (r=0.648., p<0.01), $SO_4^{2-}$ (r=0.801, p<0.01), and $NH_4^{+}$ (r=0.892, p<0.01).

Hoekstra *et al.* (2002) found high and significant inverse correlation of EC with RRG and GI similar to the present study. In his study, $NH_4^{+}$ was significantly and positively correlated with RRG and GI which was not observed in the present study.

**Table 2: Pearson correlation coefficients among various parameter of CAEs treated with in-**

| | pH | EC | $Cu^{2+}$ | $Cr^{2+}$ | $Fe^{3+}$ | $Zn^{2+}$ | $Ni^{2+}$ | $Na^{+}$ | $SO_4^{2-}$ | $Ca^{2+}$ | $NH_4^{+}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RSG%** | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | -0.616* | *ns* | *ns* |
| **RRG%** | *ns* | -0.887** | *ns* | -0.521* | -0.717** | -0.738** | *ns* | -0.924** | -0.592* | -0.777** | -0.712** |
| **GI%** | *ns* | -0.841** | *ns* | -0.555* | -0.634* | -0.713** | *ns* | -0.889** | -0.671** | -0.734** | -0.717** |
| **pH** | - | *ns* | 0.645** | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| **EC** | | - | *ns* | 0.612* | 0.822** | 0.828** | *ns* | 0.947** | 0.639* | 0.738** | 0.779** |
| **$Cu^{2+}$** | | | - | 0.641* | *ns* | 0.558* | *ns* | *ns* | *ns* | *ns* | *ns* |
| **$Cr^{2+}$** | | | | - | *ns* | 0.556* | *ns* | 0.524* | *ns* | *ns* | 0.543* |
| **$Fe^{3+}$** | | | | | - | *ns* | *ns* | 0.768** | 0.620* | 0.761** | 0.792** |
| **$Zn^{2+}$** | | | | | | - | 0.525* | 0.766** | *ns* | *ns* | *ns* |
| **$Ni^{2+}$** | | | | | | | - | *ns* | *ns* | *ns* | *ns* |
| **$Na^{+}$** | | | | | | | | - | 0.703** | 0.878** | 0.805** |
| **$SO_4^{2-}$** | | | | | | | | | - | 0.739** | 0.911** |
| **$Ca^{2+}$** | | | | | | | | | | - | 0.859** |
| **$NH_4^{+}$** | | | | | | | | | | | - |

Note: Values with ** indicates that the correlation is significant at p<0.01 level and * indicates that the correlation is significant at p<0.05; Blue colour represent the positive correlation and orange colour represent the negative correlation.

**Table 3: Pearson correlation coefficients among various parameter of CAEs treated without inoculum**

| | pH | EC | $Cu^{2+}$ | $Cr^{2+}$ | $Fe^{3+}$ | $Zn^{2+}$ | $Ni^{2+}$ | $Na^{+}$ | $SO_4^{2-}$ | $Ca^{2+}$ | $NH_4^{+}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RSG%** | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| **RRG%** | *ns* | -0.725** | *ns* | *ns* | -0.734** | *ns* | *ns* | -0.789** | -0.780** | -0.753** | -0.755** |
| **GI%** | *ns* | -0.778** | *ns* | *ns* | -0.766** | -0.544* | *ns* | -0.808** | -0.800** | -0.722** | -0.807** |
| **pH** | - | *ns* | *ns* | *ns* | *ns* | 0.577* | *ns* | *ns* | *ns* | *ns* | *ns* |
| **EC** | | - | *ns* | 0.625* | 0.861** | 0.785** | 0.517* | 0.910** | 0.801** | 0.648** | 0.892** |
| **$Cu^{2+}$** | | | - | 0.572* | *ns* | 0.581* | *ns* | *ns* | *ns* | *ns* | *ns* |
| **$Cr^{2+}$** | | | | - | *ns* | *ns* | *ns* | 0.637* | 0.608* | 0.648** | 0.744** |
| **$Fe^{3+}$** | | | | | - | 0.598* | 0.587* | 0.896** | 0.834** | 0.644** | 0.757** |
| **$Zn^{2+}$** | | | | | | - | *ns* | 0.551* | *ns* | *ns* | 0.630* |
| **$Ni^{2+}$** | | | | | | | - | 0.599* | *ns* | *ns* | *ns* |
| **$Na^{+}$** | | | | | | | | - | 0.950** | 0.874** | 0.948** |
| **$SO_4^{2-}$** | | | | | | | | | - | 0.914** | 0.912** |
| **$Ca^{2+}$** | | | | | | | | | | - | 0.888** |
| **$NH_4^{+}$** | | | | | | | | | | | - |

Note: Values with ** indicates that the correlation is significant at $p<0.01$ level and * indicates that the correlations significant at $p<0.05$; Blue colour represent the positive correlation and orange colour represent the negative correlation.

## CONCLUSIONS

Determination of the phytotoxicity in the spent mushroom substrate compost is important for guaranteeing the quality. The seed germination of *R. sativus* in CAEs of different compost mixtures amended with nitrogenous additives and inoculum was not inhibited by any concentrations except non-inoculated *Pueraria* amended SMSC. The higher GI in treatments compared to the control indicated the phyto-stimulant effect. The seed germination of *Tithonia* amended SMSC was not inhibited by any of the concentrations used in this study indicating the higher usability as an amendment in SMS composting. Further, the inoculated SMSC showed higher performance with higher GI compared to non-inoculated SMSC which may be due to the reduction of phytotoxic substances in the compost products. It can be concluded that the SMS integrated with nitrogenous additives and inoculum has the possibility to be deployed as free phytotoxic materials. Large-scale compost generation by SMS and field tests should be conducted as future research to observe crop growth and yield performance.

## AUTHOR CONTRIBUTION

SRA conceptualized and designed the study. SRA performed the experiments. SRA and WMCSJ analyzed and interpreted the data. SRA drafted the manuscript. SRA and WMCSJ critically revised the manuscript.

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