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RESEARCH ARTICLE

Effects of *Pleurotus* spp. spent mushroom substrate from NPK-rich tree leaves on growth and bioactive compounds of *Centella asiatica* L.

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Abstract

Spent mushroom waste (SMW), a by-product of mushroom cultivation, is increasingly recognized for both its environmental impact and potential for resource recovery. Consequently, the effective recycling and valorization of SMW has become essential for the sustainable advancement of the mushroom industry, particularly within the context of circular economy principles. In this study, *Pleurotus* spp. were cultivated on paddy straw as the basal substrate, amended with nitrogen, phosphorous and potassium-rich tree leaves from *Swietenia macrophylla* (SM), *Gliricidia sepium* (GS) and *Sesbania grandiflora* (SG). The study assessed the effects of these amendments on the key properties of SMW, including moisture content, dry matter, electrical conductivity (EC), pH, ash, carbon, nitrogen, protein contents and the C:N ratio, to evaluate its suitability as a natural fertilizer and soil amender for medicinal crops. The basal substrate was supplemented with different proportions (25 %, 50 %, 75 % and 100 %) of SM, GS and SG to identify the most effective substrate composition for *Pleurotus* cultivation. In addition, this study also investigated the usefulness of SMW (obtained in the production of *Pleurotus* spp.,) in the pot cultivation of *Centella asiatica* (*C. asiatica*). It examined growth, plant biomass, photosynthetic pigments and triterpene content of plants grown in four pot sets viz., 100 % casing substrate derived SMW, 75 % sand and soil: 25 % casing substrate derived SMW, 50 % sand and soil: 50 % casing substrate derived SMW and 25 % sand and soil: 75 % casing substrate derived SMW. The results showed that the 25 % sand and soil + 75 % SMW treatment significantly ($p < 0.05$) enhanced plant growth, biomass production and triterpene accumulation in *C. asiatica*. The study highlights a new, environmental-friendly strategy to increase medicinal plant productivity and bioactive compound content by using SMW derived from *Pleurotus* spp.

Keywords: *Centella asiatica* L; growth; *Pleurotus* spp.; spent mushroom waste; triterpene saponins

Introduction

Centella asiatica (L.) Urban, commonly referred to as Gotu kola, is a widely used traditional herbal medicine, particularly prevalent in Southeast Asia. It is extensively cultivated in several Asian countries, including India, Sri Lanka, China, Indonesia and Madagascar, owing to its ability to grow abundantly across diverse ecological regions (1). Numerous scientific investigations have demonstrated the benefits of *C. asiatica* and its constituents in various health issues. Its potent medicinal properties include anti-inflammatory, anti-ulcer, antipsoriatic, wound healing, anticonvulsant, immunostimulant, antidiabetic, antitumor, antibacterial and antiviral activities. Additionally, it exhibits sedative, cytotoxic, cardioprotective, insecticidal, antioxidant, antifungal and hepatoprotective effects and is also used in the treatment of leprosy and venous deficiency (2, 3).

The primary active compounds in *C. asiatica* are saponins and pentacyclic triterpenoids, including asiatic acid, madecassic acid, madecassoside and asiaticoside. This plant is highly valued for its extensive applications in traditional medicine and its popularity is growing in response to increasing global interest in sustainable, natural therapeutics. Consequently, there is rising demand for *C. asiatica*, compelling farmers to improve production yields.

Although the application of chemical fertilisers and pesticides remains a common method to boost crop productivity, such conventional farming practices present significant challenges to sustainable cultivation (4, 5).

These approaches can have adverse environmental and human health implications, thereby necessitating alternative strategies such as the use of organic fertilisers. One such alternative is SMW, a by-product of commercial mushroom production. SMW consists of the residual biomass left after mushroom harvesting (6).

Globally, it is estimated that 10 to 50 million metric tonnes of spent mushroom compost (SMC) are produced annually. The bulkiness of SMC makes disposal more labour-intensive. If not appropriately regulated, the disposal of SMC can lead to land, water and air pollution and may cause

disturbances to surrounding area (7). Therefore, it is critical to investigate and determine beneficial application rates and maximise yields while minimising chemical fertilisation.

Due to its high content of organic matter, macronutrients and a full range of micronutrients, including copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), molybdenum (Mo) and boron (B), they stand out as an exceptional choice for cultivation. SMW has been effectively used for cultivation due to its ability to neutralise soil acidity while providing a gradual release of nutrients, ensuring that crops are nourished without the risk of burning (8-11). Furthermore, the incorporation of SMW facilitates cultivation even in problematic soils (7). It serves as a reliable source of plant nutrients owing to its generally high nutrient content, slow mineralisation rate and high cation exchange capacity. Additionally, they contribute to enhancing the biological quality of crop yields (12).

The SMW of three oyster mushroom species has been reported as a fertilizer for growing *Spinacia oleracea* (13). It was also used as a media replacement for peat moss in the production of Kai-lan/Kale (*Brassica oleracea* var. *alboglabra*) in Malaysia (14). In addition, the reuse of SMW for the cultivation of *Pleurotus ostreatus* and *Pleurotus florida* has also been documented (15). SMW was also used for the tomato *Lycopersicon esculentum* mill. seedling production (16).

This study aims to evaluate the potential reuse of SMW in the cultivation of medicinal plants, with a particular focus on *C. asiatica*. Despite increasing interest, there remains limited published work on the application of SMW for enhancing the growth, yield and pentacyclic triterpenoids content in medicinal plants, which forms the basis of this study. As biometric characteristics and the concentration of bioactive compounds are primary indicators of quality and therapeutic value in medicinal plant (17), this study evaluates the suitability of SMW as a medium supplement in pot cultivation of *C. asiatica*. Taking into account the above considerations, we hypothesised that fertilisation using SMW (supplemented with nitrogen, phosphorous and potassium-rich tree leaves) would contribute to obtaining satisfactory yields of *C. asiatica*, which would allow us to consider this as fertiliser to be suitable for use in the cultivation.

Materials and Methods

Cultivation of *Pleurotus* spp.

The strain of *Pleurotus* spp. used in this study was acquired from the Regional Agricultural Research Station's, Division of Microbiology, Mushroom Research Laboratory in Tirupati, India. The culture was maintained on potato dextrose agar (PDA) slants and subcultured regularly to ensure viability. Sorghum grains were treated with the fungal culture to prepare the spawn. The grains were boiled in a water bath for 15 min and then they were mixed with calcium sulphate (2 % w/w) and calcium carbonate (4 % w/w). 300 g of grain were placed into 40 microns thick polythene bags with a 1000 g capacity. These bags were sterilized by autoclaving for 30 min at 15 psi (pounds per square inch) and then allowed to cool to room temperature. Once cooled, the sterile grains were inoculated with the mother spawn and incubated for 20 days at 28±2 °C. During this period, the grains were completely covered by white mycelium to create mushroom beds.

The cultivation of *Pleurotus* spp. followed a modified version of the method (18). Leaves from SM, GS and SG were mixed with well-dried paddy straw on a dry weight basis in different proportions (25 %, 50 %, 75 % and 100 % v/v plant leaves residues). This mixture was chopped into pieces that were 2-3 cm long and soaked overnight. After draining the excess water, the slightly moist casing substrate was sterilized for 30 min at 121 °C and then cooled to room temperature. The casing substrate was inoculated with 30 g of spawn per kg of substrate.

The inoculated beds were punctured to allow air exchange and incubated in a dark chamber at 28±2 °C and 70±5 % relative humidity for 15 days. subsequently, the fully colonised beds were transferred to a cropping room maintained at 24 ± 2 °C and ≥90 % relative humidity. Watering was performed twice daily; however, irrigation was withheld one day before the initial harvest. The first flush of mushroom fruiting bodies was harvested 25 days post-inoculation. Subsequent harvests occurred biweekly over a 60-day cropping cycle.

Characterisation of SMW

After 60 days of cultivation, the SMW, derived from *Pleurotus* spp. grown on substrates amended with varying proportions (25 %, 50 %, 75 % and 100 % v/v) of SM, GS and SG plant residues, was dried in the dark for two days at 50 °C and then crushed into particles (about 5 mm in diameter). Prior to seeding, the physicochemical properties of the SMW were determined. The nitrogen content and proteins were calculated using the following formula (19):

$$\text{Proteins \%} = \text{Nitrogen \%} \times 6.25 \quad \text{(Eqn. 1)}$$

Moisture content, ash content and carbon content were calculated using the method (20). The formula (density of wet SMW - the density of dry SMW) was used to determine the compacting factor (21). pH and EC of SMW were measured in distilled water at a ratio of 1:10 (w/v) ratio (22).

Cultivation of plants - experimental design

The *C. asiatica* nursery was grown in plastic pots (10.2 cm × 25.4 cm) that were disinfected using 70 % ethanol. Each pot was filled with a potting medium consisting of 1 kg of a sand and sterilized garden soil mixture (1:1 v/v), supplemented with 1.0 kg of casing substrate derived SMW in various ratios (25 %, 50 %, 75 % and 100 % v/v). After soaking in a 2 % NaOCl solution for 10 min, the stem cuttings with a single node (2-3 cm) were surface sterilized and cultivated on potting media. The following treatments were applied to the plantlets after they were planted in four distinct pot sets: T1: 100 % SMW; T2: 25 % SMW + 75 % soil: sand mix; T3: 50 % SMW + 50 % soil: sand mix and T4: 75 % SMW + 25 % soil: sand mix.

According to a completely randomized design, 24 plants in total, each in a different poly pot, were created, with six experimental replicates made for each treatment. Under natural photoperiods (23.5/18 °C day/night, 4000-6000 lux light intensity, 16/8 hr light-dark cycle and 55/75 % relative humidity), the plantlets were cultivated in a glasshouse and received daily watering. Plants were harvested 60 days after planting.

Analysis of biometric parameters and chlorophyll content

On the 60th day following planting, the biometric data of the experimental plants under each treatment were recorded.

Using a standard scale, the shoot length of the plants was measured from the collar region to the tip of the plant after they were gently removed without harming the root system. The number of fully opened leaves per plant was manually counted. Petiole length, leaf length and leaf width were measured in centimetres using a measuring scale for each treatment. The number of nodes along each primary branch was also manually recorded.

After the plants were gently removed, any leftover dirt particles were washed away from their roots under running water. The entire plant was then blotted dry using blotting paper, after which the shoot region was separated from the root system. A monopan electronic balance was used to measure the fresh weight of the roots and shoots after they had been blotted dry on blotting paper and recorded in g. Following the measurement of the fresh biomass of the roots and shoots, the plants were wrapped in paper and dried for two days at 60 °C in a hot air oven to get a consistent weight to compute the dry weight in g.

After being removed from the plants, the leaves were cleaned with distilled water and patted dry. In a mortar, one g of leaf material was homogenized with 80 % acetone. To make grinding easier, a small amount of $CaCO_3$ was added. The resultant solution was then centrifuged for 15 min at 5000 rpm and the supernatant was diluted with 80 % acetone to a final volume of 10 mL. After passing the clear supernatant through Whatmann No. 1 filter paper, it was placed in a 1 cm glass cuvette. Using 80 % acetone as a blank, the absorbance was determined using the specific absorption coefficient for chlorophyll a and b at 645 nm and 663 nm in a Shimadzu (UV-1800) spectrophotometer. Chlorophyll concentrations were measured using the specified equations (23).

Extraction of triterpene saponins

The amount of triterpene saponins in the leaves of each treatment was accurately ascertained following the established protocol (24). *C. asiatica* leaves were separated and dried for three to four days at 50 °C in an oven. A small grinder was used to grind the dried samples into a powder. One g of the powder was transferred to sterile, screw-capped bottles with a wide mouth for solvent extraction. After soaking for 24 hr at room temperature, it was heated at 100 °C for an hr.

After that, the mixture was centrifuged for 10 min at 4 °C at 2000 rpm. A syringe filter with a 0.2 µm cellulose acetate membrane was used to filter sterilize the supernatants after they had been filtered through a sterile funnel containing sterile Whatman filter paper no. 1 (25). To defat the filtrate, petroleum ether was applied three times for one hr at 40°C. After filtering out the petroleum ether, the sample was subjected to methanol extraction, carried out three times for one hr each under mild heating.

The combined methanol extracts were concentrated and dissolved in a methanol-acetone mixture (1:5 v/v) to precipitate the saponins (25). The resulting white, amorphous precipitate, referred to as the crude extract (CE), was vacuum-dried. The CE was then applied to a silica gel-60 chromatography column (230-400 mesh, Merck) and eluted with a chloroform-methanol-water mixture (70:30:10) (25). The pure saponin fraction was obtained by collecting and evaporating the first fraction at a low temperature, leaving behind the saponin-rich residue.

High-performance liquid chromatography (HPLC) analysis of triterpene saponins

The dried saponin extract was dissolved in 10 mL of methanol. Three distinct concentrations of triterpene standards were made, provided by Dr. D. Pragathi from the Department of Biotechnology of Sri Venkateswara University, Tirupati. A stock solution of asiatic acid (2.5 mg/mL) was diluted with HPLC-grade methanol to obtain concentrations of 0.25, 1.0 and 2.5 mg/mL. Similarly, madecassoside and asiaticoside were diluted from a stock solution (5.0 mg/mL) to yield final concentrations of 0.5, 2.5 and 5.0 mg/mL, respectively. The extract solution was also diluted using methanol at a 1:5 (v/v) ratio. Prior to injection, both the standards and the samples were filtered using a 0.45 µm Whatman filter.

The HPLC system consisted of a Waters separation module (Waters, Milford, MA, USA), equipped with a UV-VIS spectrophotometric detector (model 484), an autoinjector (model U6K) and a pump (model 501), all controlled via Empower chromatography data software. Chromatographic separation was performed on a reversed-phase RP-18 LiChroCART® column (250 mm × 4 mm I.D.; particle size: 5 µm). The mobile phase employed an acetonitrile–water gradient, with a flow rate of 1 mL/min and detection was carried out at a wavelength of 206 nm. For each sample and standard, triplicate injections of 20 µL were performed.

Triterpene concentrations in the samples were determined by plotting the peak area against the concentration of the standards; madecassoside, asiaticoside and asiatic acid; using calibration curves with correlation coefficients (r^2) greater than 0.99. Results are expressed in mg/mL.

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) in the Statistical Package for the Social Science (SPSS) software, version 11.5. Results are expressed as mean ± S.D. A p-value < 0.05 was considered statistically significant.

Results and Discussion

Characterisation of casing substrate and spent mushroom substrates

Table 1 presents the physicochemical characteristics of the casing substrates prepared using SM, GS and SG plant leaves combined with well-dried paddy straw (on a dry weight basis) in varying amounts (25 %, 50 %, 75 % and 100 % v/v plant leaf residues). The moisture level of 25 % casing substrate was higher at 67.69 %, followed by 75 % substrate at 66.45 % and 50 % substrate at 63.83 %. The moisture content of 100 % substrate then dropped to 62.12 %. The 100 % casing substrate had a significantly higher dry weight (25.21 %), followed by 50 %, 75 % and 25 % casing substrates (23.49 %, 23.11 % and 22.54 % respectively).

On the other hand, the substrates' pH values ranged from (6.42-6.64) exhibiting a slightly acidic condition. EC was used to estimate total salt content and was reported as 2.13, 1.91 and 1.88 ms/cm for 100 %, 75 % and 25 % casing

Table 1. Physicochemical properties of different proportions of casing substrate before *Pleurotus* spp. mushroom cultivation

Properties	25 % casing substrate	50 % casing substrate	75 % casing substrate	100 % casing substrate	F value (p<0.05)
pH	6.64	6.53	6.59	6.42	4.65
EC (ms/cm)	1.88	1.75	1.91	2.13	165.94
Moisture content %	67.69	63.83	66.45	62.12	7.01
Dry matter %	22.54	23.49	23.11	25.21	3.87
Ash %	15.98	14.71	14.32	14.56	15.27
Carbon (g/kg)	303.25	314.37	292.11	265.63	73.11
Nitrogen (g/kg)	15.18	12.83	16.05	10.96	60.54
C: N Ratio	19.97	24.50	18.20	24.23	18.22

substrates and 1.75 ms/cm for 50 % casing substrate. The 50 % casing substrate had the substantially greater carbon content (314.37 g/kg) than the 25 %, 75 % and 100 % casing substrates, which had lower carbon contents (303.25, 292.11 and 265.63 g/kg respectively). The nitrogen content was highest in the 75% substrate (16.05 g/kg) and comparatively lower in the 25 % (15.18 g/kg), 50 % (12.83 g/kg) and 100 % (10.96 g/kg) substrates. The C:N ratio was lowest in the 75 % substrate (18.20), followed by the 25 % (19.97), 100 % (18.20) and 50 % (24.50) substrates. The ash content was highest in the 25 % substrate (15.98 %) and slightly lower in the 50% (14.71 %), 75% (14.32 %) and 100% (14.56 %) casing substrates.

According to Table 2, the type of carbon source significantly influenced weight loss during cultivation. The highest weight loss was observed in the 100 % casing substrate-derived SMW (15.51 %), followed by the 75 % (11.94 %), 25 % (10.97 %) and 50 % (9.19 %). The compacting factor after *Pleurotus* spp. cultivation reached 0.13 and 0.11 for the 100 % and 75 % casing substrate-derived SMW, respectively, but declined to 0.08 and 0.05 for the 25 % and 50 % . The increase in compacting factor correlated with higher SMW density across all substrate types prior to mushroom cultivation, which negatively impacted mycelial growth. This factor primarily depends on substrate type and fungal strain (26).

The pH of SMW extracts was found to be acidic, averaging 5.18. The highest pH values were recorded for 25 % and 75 % casing substrate derived SMW (5.36 and 5.28 respectively), whereas 100 % casing substrate derived SMW had lesser pH (4.99), followed 5.11 for 50 % casing substrate derived SMW. Similar results were observed in previous study where *P. ostreatus* grown on wheat straw showed a decrease in SMW pH from an initial 5.80-5.90 to a final range of 4.62-4.82 (27, 28). This decline may be due to organic acid accumulation from phosphate solubilisation (29) or byproducts/metabolites of mushroom (30). Similarly, a previous study reported a drop in PDA medium pH from 6.8-5.8 following *P. ostreatus* growth (31). Another study noted a reduction in PDB medium pH from 7.0 to approximately 5.27-4.59 after nine days from oyster mushroom inoculation (32).

100 % casing substrate derived SMW showed the higher EC 2.31 ms/cm, while the lower value reached to 1.97 ms/cm for 50 % casing substrate derived SMW; these observed values were in the range considered optimal for edible mushroom and plants production (33). The washing of SMW to reduce EC or mixing it with low-EC substrates to enhance reusability. A lower EC generally contributes to improved agronomic performance (34).

Table 2 also illustrates the carbon content across SMW treatments which reflects the influence of cellulosic composition. The 50 % SMW had the highest carbon content (298.49 g/kg), while the 100 % SMW had the lowest (246.11 g/kg). These values were lower than those of uncultivated substrates, likely due to CO_2 release during fungal decomposition via exo-enzymes (35).

The higher nitrogen content of SMW was recorded as 8.47 g/kg for 50 % casing substrate derived SMW followed by 7.15 and 7.03 g/kg for 25 % and 75 % casing substrate derived SMW respectively. 100 % casing substrate derived SMW had lower content 6.72 g/kg. The casing substrate derived SMW of different proportions prepared in the current study reported higher percentage of the protein content at average 7.45 %. A previous study reported a similar finding where the protein content was found to be 7.88 % (36). The highest C:N ratio was found in the 25 % casing substrate derived SMW at value 39.76, while 50 % casing substrate derived SMW had considerably lower ratio (35.24). These results agree with the previous findings that referred the optimum C/N ratio for oyster mushroom *P. ostreatus* was 35.2. A high C/N ratio could enhance the digestibility of the lignocellulose content, followed by high availability of cellulose materials as mushroom nutrients (37).

Ash content in SMW was lowest in the 25 % treatment (10.19 %) and highest in the 50 % treatment (12.61 %), consistent with the values (12.80-18.25%) for *P. ostreatus* cultivated on wheat straw substrates (28, 36).

Table 2. Physicochemical properties of different proportions of SMW before the cultivation of *C. asiatica*

Properties	25 % casing substrate derived SMW	50 % casing substrate derived SMW	75 % casing substrate derived SMW	100 % casing substrate derived SMW	F value (p<0.05)
Losing weight (%)	10.97	9.19	11.94	15.51	113.12
Compacting factor	0.08	0.05	0.11	0.13	14.70
pH	5.36	5.11	5.28	4.99	14.48
EC (ms/cm)	2.08	1.97	2.15	2.31	115.95
Carbon content (g/kg)	284.34	298.49	270.96	246.11	76.99
Nitrogen content (g/kg)	7.15	8.47	7.03	6.72	81.33
C:N ratio	39.76	35.24	38.54	36.62	9.30
Protein (%)	7.72	7.95	7.33	6.82	75.46
Ash (%)	10.19	12.61	11.48	12.23	85.30

Analysis of biometric parameters and chlorophyll content

There was a significant variation (p<0.05) in shoot length of *C. asiatica* plants treated with 25 % garden soil + sand: 75 % casing substrate derived SMW when compared with the other treated groups (Table 3 and Fig. 1). The T4 treated group recorded the highest shoot length (42.13±2.88 cm) at the 60 day harvest in *C. asiatica*. The individual treatments did not differ significantly with each other. The root length of *C. asiatica* plants varied in T4 treatment when compared to T1, T2 and T3 treated plants. The plants grown under T4 treatment exhibited maximum root length (17.56±2.03 cm) and the root length was found minimum (12.15±1.59 cm) in T1 treatment on 60 days of plant growth (Table 3 and Fig. 1).

The observed shoot elongation corroborates findings in *Capsicum annuum* L., where application of SMW, both fresh and as leachate; significantly enhanced shoot height after 35 days, attributed to increased nutrient availability and uptake (38). Similarly, SMW significantly increased root length in baby spinach, with higher rates of application resulting in longer roots. This effect is likely due to the loose texture, high organic matter and improved water retention properties of SMW, which create favourable conditions for root expansion (39).

Leaf number significantly increased in the T4 group (5.83±0.39, p<0.05) compared to T1, T2 and T3 (Table 3 and Fig. 1). In *C. annuum* L., SMW treatment notably increased branching and leaf number (38). In a different study, the highest number of leaves (19.67 per plant) was obtained by applying SMW (15 %) and sulfur (3000 kg/ha) together (40). This study demonstrated that, when combined with complementary amendments, SMW significantly contributes to the growth of leaves. In the current study, the increase in leaf count, is consistent with the study reported by (38, 40) and may be attributed to enhanced phosphorus availability, enriched microbial diversity and improved water retention and aeration.

Petiole length was highest in T4, followed by T3, T2 and T1 (Table 3 and Fig. 1). The shortest petiole length (3.48±0.27 cm) was recorded in the non-inoculated control plants. The study on strawberry plants, which are commonly used as a model for studying how plants grow, found that using SMC, a type of SMS made the petiole length much longer. In controlled trials, the group with 100 % SMC had the longest petiole length, followed by combinations with larger amounts of SMC. The statistical study showed that these changes were real, which means that SMC can help plants' petioles grow longer. SMC can make macro and micronutrients more available, which is why the petiole length has increased. SMC may also help create a rich microbial community, which includes helpful groups like *Trichoderma* spp. and *Pseudomonas* spp. These species can help plants thrive and fight off infections (41).

Leaf length and width were influenced by casing substrate derived SMW (Table 3 and Fig. 1). The T4 treatment group showed in the highest values for both leaf length and leaf width (3.51±0.57 cm and 4.19±0.37cm respectively), exceeding those in T3, T2 and T1 treatments. Basil, a commonly used medicinal plant, showed marked improvements in leaf length and leaf width when SMW from *P. ostreatus* was applied as a soil amendment. SMW treatments, either by themselves or in conjunction with earthworm humus and organic compost, increased leaf area, which was directly correlated with improvements in leaf length and width. It was consistently observed in this study that SMW-amended soils supported greater leaf expansion than controls when leaf area was measured at various intervals (15, 30, 45 and 60 days after transplanting) (42). It has been reported that SMW may provide nitrogen, phosphorus, potassium and improves soil physical properties, which together promotes robust leaf development. Research on other crops and medicinal plants demonstrates that SMW amendments can improve soil microbial activity, water retention and aeration, thereby increasing leaf size, including both length and width (43).

Node count was highest in the T4 (6.45±0.45) compared to T1 (3.80±0.57), with all treatments showing a statistically significant increase (p<0.05) (Table 3 and Fig. 1). T4 outperformed all other treatment, followed by T3 (5.97±0.84) T2

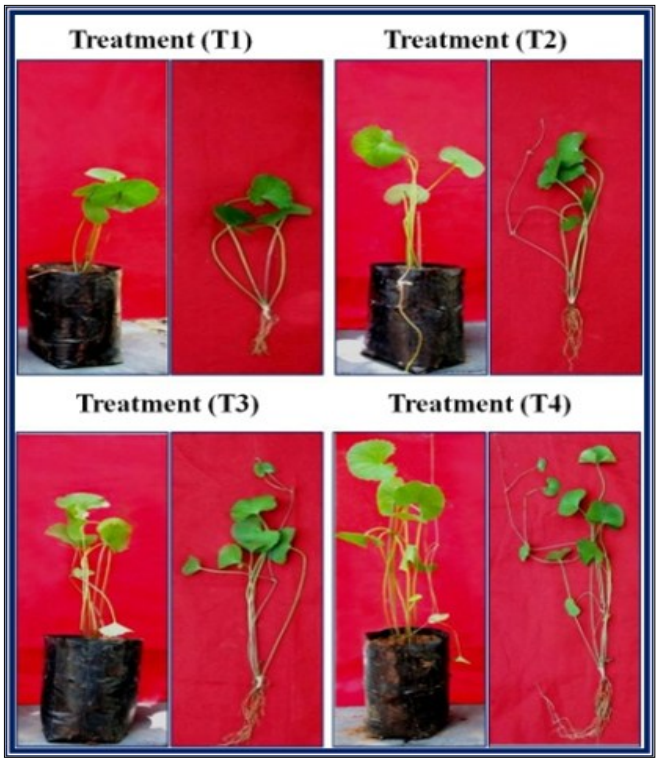

Fig. 1. Biometric parameters of *C. asiatica* upon the cultivation with SMW derived from *Pleurotus* spp.

Table 3. Influence of SMW on biometric parameters of *C. asiatica*

Parameters/Treatments	T1	T2	T3	T4	F Value (p<0.05)
Shoot length (cm)	33.90±3.17	37.45±2.51	39.51±2.37	42.13±2.88	9.57
Root length (cm)	12.15±1.59	15.75±2.10	16.01±2.15	17.56±2.03	8.01
Petiole length (cm)	3.52±0.22	4.99±0.54	5.39±0.36	5.99±0.57	33.42
No. of leaves	3.01±0.53	4.37±0.42	4.96±0.54	5.83±0.39	37.38
Leaf length (cm)	2.28±0.29	2.90±0.82	3.12±0.34	3.51±0.57	5.30
Leaf width (cm)	2.65±0.21	3.43±0.28	3.91±0.17	4.19±0.37	37.83
No. of nodes	3.80±0.57	5.55±0.38	5.97±0.84	6.45±0.45	23.24
Total fresh biomass (mg)	6.19±1.28	7.84±1.45	9.99±1.72	11.20±1.39	13.76
Total dry biomass (mg)	4.98±0.91	5.36±0.74	5.86±0.56	7.74±0.82	15.23

(5.55±0.38) and T1. In *Capsicum chinense* (a medicinal plant), SMW application (600 g SMW per 6 kg soil) significantly increased leaf retention and reduced leaf drop, correlating with an increase in node number (44). Similarly, *Corchorus olitorius* grown in 20 % SMW-amended soil exhibited optimal leaf production and stem girth, suggesting robust nodal development. It was reported that the nitrogen, phosphorus and potassium of SMW are critical for meristematic activity at stem nodes. For instance, nitrogen in SMW supports phytohormone synthesis (e.g., cytokinins), promoting lateral bud activation and node formation (44).

The effects of casing substrate derived SMW on plant growth is shown in Table 3 and Fig. 1. Total fresh biomass of *C. asiatica* varied from 6.19±1.28 gm (in T1) to 11.20±1.39 gm (in T4). All treatments showed statistically significant effect (p<0.05). The greatest increase in total dry biomass (7.74±0.82) was recorded in T4 compared to T3, T2 and T1. The treatment comprising 25 % garden soil and sand with 75 % casing substrate derived SMW significantly (p<0.05) outperformed the remaining ones as for total dry biomass. It has been shown that treating 50–75 % SMW particularly that derived from *P. ostreatus*, significantly increased the fresh and dry biomass of *Ocimum basilicum*. Additionally, compared to control treatments, plants demonstrated a notable increase in essential oil yield. The effect on the overall growth and yield was most noticeable when SMW was applied either alone or in 75:25 and 50:50 ratios with organic compost (42). The high nutrient content of SMS being rich in nitrogen, phosphorus and potassium enhances soil fertility, structure and nutrient availability, leading to greater accumulation of biomass in medicinal plants (43). However, minimal quantities of SMW (up to 25 %) in the growth medium for *Gossypium herbaceum* and *Talinum paniculatum* enhanced biomass accumulation, whereas elevated levels (75–100 %) resulted in reduced growth or plant mortality, likely attributable to nutrient imbalances and increased EC (45).

The use of casing substrate derived SMW has enhanced chlorophyll a and chlorophyll b from 5.89±0.17 and 2.81±0.11 in T1 to 9.95±0.26 and 4.10±0.17 in T4 respectively (Table 4). Among all, T4 showed the highest effect, followed by T3, T2 and T1. Total chlorophyll was highest in T4 (13.97±0.28), followed by T3 (11.95±0.37), T2 (10.63±0.22) and T1 (8.73±0.23) (Table 4). Similar patterns were observed in tomato seedlings and basil (*Ocimum basilicum*), where adding 5-15 % SMS increased the levels of chlorophyll a and b, which were correlated with higher biomass and leaf area. Accumulation of phosphorus and nitrogen in the growing media by SMW, as well as by increased microbial activity promote the nutrient mineralization. Tomato seedlings exposed to 50 % to 100 % SMW rates showed reduced chlorophyll content and observable chlorosis symptoms. The main causes of these adverse effects are increased EC and possible accumulation of toxic material in the growth medium (46, 47). These observations highlight that the optimal application rate is plant species-specific and should be moderated to avoid adverse effects.

The concentration of madecassoside, asiaticoside and asiatic acid contents of *C. asiatica* were found to be maximum in T4 treatment (2.21±0.07, 1.99±0.05 and 0.06±0.002 mg/mL respectively), followed by T3 (2.09±0.05, 1.81±0.04 and 0.06±0.001 mg/mL), T2 (1.98±0.06, 1.66±0.05 and 0.05±0.003 mg/mL) and T1 (1.79±0.04, 1.58±0.03 and 0.03±0.002 mg/mL) (Table 4 and Fig. 2). The application of casing substrate derived SMW significantly enhanced triterpene saponins content in *C. asiatica* across treatments (Table 4 and Fig. 2). Previous studies have shown that SMS-derived extracts (e.g., warm water extracts) stimulated lettuce seedling growth by 27 %, linked to enhanced uptake of nutrients (nitrogen, phosphorus and potassium) and upregulation of antioxidant-related genes (*ggps*, *hppd*, *pdx1*) involved in vitamin E and B6 biosynthesis (48). Further, evidence supports similar mechanisms that apply to medicinal plants through the enhanced nutrient uptake and the synthesis of secondary metabolites like alkaloids, phenolics and terpenoids in medicinal plants. SMS contains higher phenolic content than mushroom fruiting bodies and hence when used as a soil amendment, these phenolics may transfer to plants or induce phenolic biosynthesis pathways (49). The aforementioned studies underscore versatility of SMW in enhancing bioactive compounds through nutrient uptake and direct biochemical contribution. However, the optimal results depend on the plant species and the proportion of SMS in the growing media, with moderate inclusion rates generally providing the best outcomes.

Conclusion

The study revealed that the introduction of tree leaves that are high in nitrogen, phosphate and potassium into paddy straw based SMW significantly enhanced its efficacy as a natural fertilizer and soil amendment. The most effective combination for enhancing the growth, biomass and triterpene levels of *C. asiatica* was 25 % sand and soil and 75 % SMW of spent mushroom casing substrate. Recycling waste from the aforementioned industries into valuable products will be an excellent means to propagate the ideas of the circular economy and sustainable agriculture.

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Table 4. Influence of SMW on biochemical parameters of *C. asiatica*

Parameters/Treatments	T1	T2	T3	T4	F value (p<0.05)
Chlorophyll a (mg/g)	5.89±0.17	7.38±0.21	8.44±0.22	9.95±0.26	372.64
Chlorophyll b (mg/g)	2.81±0.11	3.27±0.16	3.41±0.19	4.10±0.17	66.60
Total chlorophyll (mg/g)	8.73±0.28	10.63±0.42	11.95±0.39	13.97±0.58	157.17
Madecassoside (mg/mL)	1.79±0.04	1.98±0.06	2.09±0.05	2.21±0.07	60.61
Asiaticoside (mg/mL)	1.58±0.03	1.66±0.05	1.81±0.04	1.99±0.05	104.32
Asiatic acid (mg/mL)	0.03±0.002	0.05±0.003	0.06±0.001	0.06±0.002	266.66

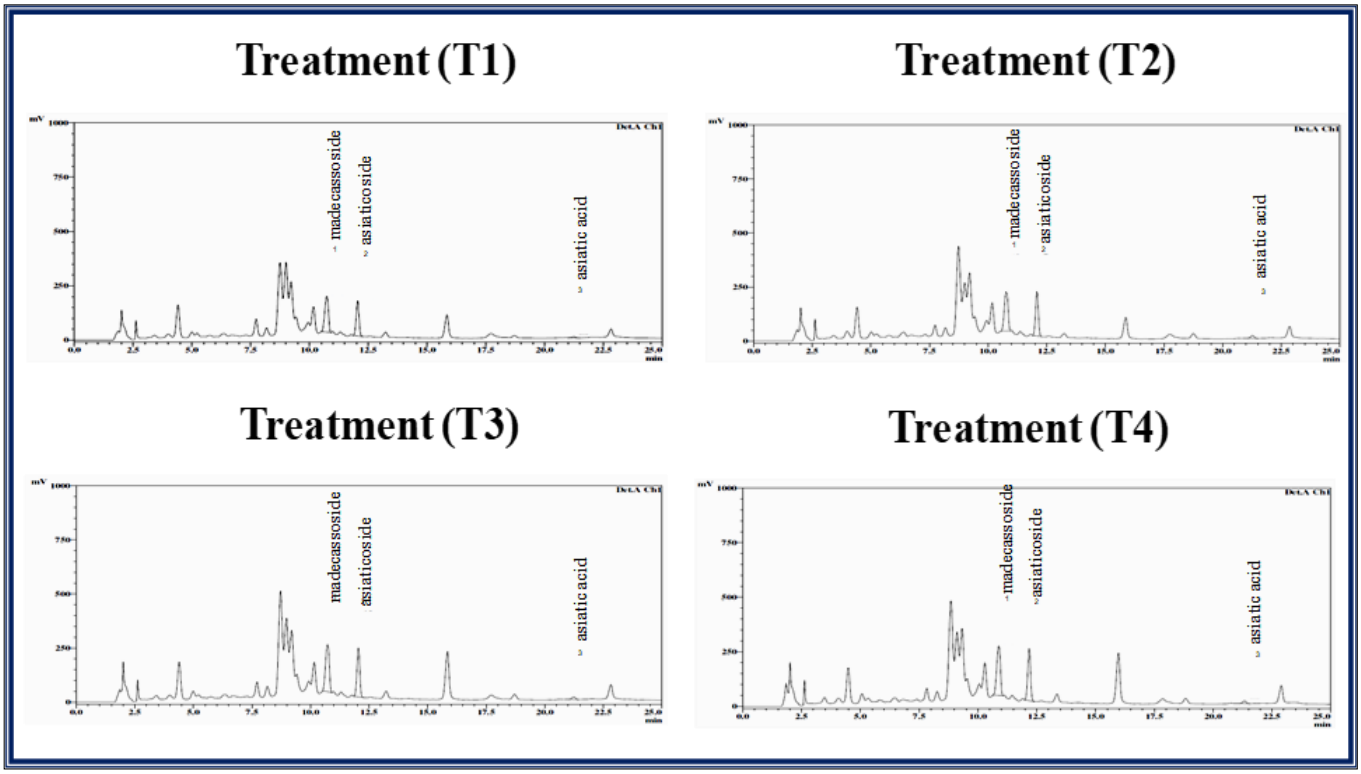

Fig. 2. High performance liquid chromatography profile of triterpene saponins extracted from plants raised from different proportions of SMW of *Pleurotus* spp.

biometric experiments on *Centella asiatica*. The authors also extend special thanks to Dr. D. Pragathi for providing the bioactive standards.

Authors' contributions

KGN conducted the experiments, performed data analysis, drafted and reviewed the manuscript. MND participated in design of experiment and reviewed the manuscript. MSPR participated in editing and refining the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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