RESEARCH ARTICLE

Azolla pinnata, Aspergillus terreus, and Eisenia fetida for faster recycling of nutrients from wheat straw

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Abstract

A vast amount of surplus wheat straw/stubble (a carbon-rich bioresource) is wasted every year by burning. Harmful gases and residue matter released due to burning cause harmful effects on the environment and human health. Therefore, there is a strong need to recycle this bioresource in a sustainable manner. In the present study, wheat straw (W) was spiked with cattle dung (C), Azolla pinnata (A), and Aspergillus terreus (F) to make eight different treatments (1 kg each), viz. W (1 kg), WC (666 g + 334 g), WA (980 g + 20 g), WF (980 g + 20 ml), WCF (666 g + 314 g + 20 ml), WCA (666 g + 314 g + 20 g), WFA (960 g + 20 ml + 20 g), and WCFA(666 g + 294 g + 20 ml + 20 g), and subjected to vermicomposting (Vcom) and aerobic composting (Acom). A comparison was made for the time required for degradation and nutrient profile of the products. The fastest recycling of wheat straw/stubble (120 days) was observed in WCA and WCFA, but the nutrient quality of WCAwas better (N 18.67, P 3.88, K 38.84 g/kg). In the Acom group, longer time was required for degradation of various mixtures, but in this group also, WCA was degraded first of all (138 days) and yielded a product with the best nutrient quality (N 14.77, P 2.56, K 28.80 g/kg). Maximum growth of E. fetida and maximum number of hatchlings were observed in WCA while the highest cocoon production was observed in WCFA. It was observed that azolla enhanced conversion of wheat straw into a nutrient-rich product for agronomic use. Thus its use will reduce the amount of cattle dung in the mixture and the bulk to be handled by the farmers for ecosafe disposal of surplus straw/stubble. Therefore, this technology can be adopted as an alternative to burning.

Keywords Wheat straw/stubble · Azolla pinnata · Cattle dung · Aspergillus terreus · Eisenia fetida

Introduction

Wheat residue ranks second among the cereal crop residues and contributes 22% to the total quantity of 325 million tons (mt). The residue left after harvesting of wheat is valued highly for animal feed and as a substrate for production of mushrooms, pulp, butanol, bihydrogen gas, biogas, and bioethanol (Sidhu et al. [1998](#page-11-0); Jimenez et al. [2000;](#page-10-0) Zhang et al. [2002;](#page-11-0) Fan et al. [2006](#page-10-0); Qureshi et al. [2007](#page-11-0); Kaparaju et al. [2009\)](#page-10-0). Despite so many uses of this straw, burning seems to be the most economical and easiest way for the farmers around the globe to get rid of wheat stubble that is left after mechanical

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harvesting (Gupta et al. [2004\)](#page-10-0). This is mainly because of lack of buyers, shortage of time for the next crop, lack of assistance from the government, high cost of labor, and general belief that burning results in increased productivity of the next crop (Ahmed et al. [2015;](#page-9-0) Arjinder [2017\)](#page-10-0). In aggregate terms, China, India, and the USA are the top burners of crop residues, followed by Brazil, Indonesia, and the Russian Federation (Memon et al. [2018](#page-11-0); Cassou [2018](#page-10-0)). Field burning by the two major contributors, i.e., India and China, accounts for wastage of 33.4% biomass (Streets et al. [2003\)](#page-11-0). Around 24.5 mt of surplus wheat straw/stubble is burnt annually on the farms of India (IARI [2012\)](#page-10-0). Punjab, the food bowl of India, produces 22.5% of the total wheat in the country (Singh and Singh [2015](#page-11-0)) and nearly 36% straw/stubble is burnt (out of total 41% produced) in the fields every year (Kumar et al. [2015\)](#page-10-0). Burning of the straw/stubble not only leads to loss of N (40–80%) but also releases greenhouse gases (GHGs) like methane, carbon dioxide, and nitrous oxide in the air and causes an increase in the earth's temperature (Tripathi [2015\)](#page-11-0). It has been reported that burning of wheat contributes about

113 Gg of CO, 8.6 Gg of NOx, 1.33 Gg of CH4, 13 Gg of PM_{10} , and 12 Gg of PM_{2.5} (Badarinath et al. [2006](#page-10-0)). It causes a decline in organic matter and beneficial nutrients in the soil, a change or decrease in the diversity and population of beneficial soil microbes, and a reduction in the enzymatic activity of microbes (Hesammi et al. [2014](#page-10-0)). Not only does burning cause various environment and health-related problems but it also reduces availability of the straw to livestock (short by 40%) (Kumar et al. [2015](#page-10-0)). Therefore, there is a strong need to harvest this C- and mineral-rich resource wisely. An ecofriendly alternative is to convert it into vermicompost, the miracle product for crops. Vermicompost is rich in NPK (nitrogen 2–3%, phosphorus 1.55–2.25%, and potassium 1.85– 2.25%), micronutrients, beneficial soil microbes like "nitrogen-fixing bacteria," and "mycorrhizal fungi" and helpful for plant growth (Sinha et al. [2010\)](#page-11-0). Vermicomposting is considered even better than composting because it is fast and nutrients in its product, being water soluble, are easily available to the plants (Sudhakar et al. [2002](#page-11-0); Aalok et al. [2009\)](#page-9-0). Vermicompost has greater fertilizer value due to less phytotoxicity and high humus content in comparison to compost (Kaur et al. [2010](#page-10-0); Lim et al. [2012](#page-10-0)).

Recycling of agro-industrial wastes using earthworms has become an important component of sustainable agriculture and has a multidirectional impact in terms of safe disposal of organic wastes. Earthworms have the ability to convert a variety of organic wastes into fine mucus-coated fecal pellets, popularly known as vermicompost. Earthworms are the natural fertilizer factories which serve as biocatalytic agents to enhance soil fertility through their physical, chemical, and biological processes. These activities of worms help in faster recycling of nutrients from a variety of plant matter in the fields. However, wheat straw/stubble is a recalcitrant bioresource in comparison to other agricultural residues as it is rich in cellulose, hemicellulose, and lignin (Khan and Mubeen [2012\)](#page-10-0) along with a high carbon to nitrogen ratio (Bakker et al. [2013\)](#page-10-0). In the present study, an attempt has been made for enhancing the rate of degradation and quality of the product from this crop residue. For this purpose, wheat straw/ stubble was amended with *Azolla pinnata* (a nitrogen-fixing weed), Aspergillus terreus (cellulolytic fungi), and cattle dung and subjected to vermicomposting (with *Eisenia fetida*) and aerobic composting for recycling of nutrients that are otherwise lost by burning. Azolla is a free-floating water fern that floats in the wastewaters, fixes atmospheric nitrogen because of its association with the nitrogen-fixing cyanobacterium Anabaena azollae (Raja et al. [2012](#page-11-0)), and is used as a biofertilizer (Yadav et al. [2014\)](#page-11-0). The beneficial effect of Azolla is that it increases the soil's organic matter, improves soil quality, and supplies fixed nitrogen. After its decomposition, humus is formed which increases aeration and drainage as well as the water-holding capacity of soil (Bhuvaneshwari and Kumar [2013](#page-10-0)). Cattle dung is a valuable fertilizer that

contains a broad range of nutrients and is an excellent source of organic matter (Gupta et al. [2016](#page-10-0)). It is produced in large amounts in India but wasted due to non-availability of space and high cost of collection. Aspergillus terreus, a fungus known to possess lignocellulolytic enzymes, helps in the degradation of the biomass rich in cellulose and lignin (Emtiazi et al. [2001](#page-10-0); Kumar and Parikh, [2015\)](#page-10-0). It was expected that azolla, cattle dung, A. terreus, and E. fetida will together reduce the time of conversion of wheat straw/stubble into a quality product. The study holds importance as no report is available till date on the use of A. pinnata, A. terreus, and cattle dung for harvesting the nutrients locked in wheat straw/stubble.

Materials and methods

E. fetida with an average weight of 0.50 g was taken from the vermifarm of Guru Nanak Dev University, Amritsar. Azolla was procured from a local village pond and cultured in rectangular pits ($10' \times 10' \times 1.5'$) at the university; each pit was lined with a polythene sheet, topped with a 2-cm layer of garden soil and cattle dung slurry, and filled with water. Pure culture of Aspergillus terreus was procured from the Department of Microbiology, GNDU, grown on potato dextrose agar (PDA), and maintained at 4 C till use. Erlenmeyer flasks having 50 ml sterile glucose broth (glucose and yeast extract and K_2HPO_4 and $MgSO_4$ at pH 7) were used for preparing the inoculum. Discs of 4 mm diameter were taken from 7-day-old culture, added to a flask with glucose broth, and kept in a rotatory shaker at 121 rpm for 42 h at 40 °C. Cattle dung was collected from nearby dairy farms, and wheat straw/ stubble was obtained from the fields on the campus, dried, and chopped (2 mm) before the experiment. The experiment was conducted in triplicate in plastic tubs ($65 \times 45 \times 30$ cm) under the sheds. Chemical characteristics of wheat straw/stubble (W), cattle dung (C), and azolla (A) were estimated prior to the start of the experiment (Table [1](#page-2-0)). The straw/stubble was spiked with cattle dung, azolla, and fungus (1 kg mixture) to make 8 different mixtures (Table [2](#page-2-0)).

The mixtures were subjected to vermicomposting (Vcom group) and aerobic composting (Acom group). In the Vcom group, 100 non-clitellate earthworms were inoculated in each tub after initial stabilization (removal of volatile toxins and heat) of 15 days. The tubs were covered with a jute mat and water was sprayed as and when required to maintain moisture. Biomass and population buildup (cocoons, hatchlings, and adults) of earthworms was recorded in the Vcom group at 20-day intervals (0, 20th, 40th, 60th, 80th, 100th, and 120th day). Worms, cocoons, and hatchlings were hand sorted, counted separately for each treatment, and put back in the respective trays afterwards. Mixtures of the Acom group were turned manually on alternate days for aeration of the waste.

Parameters	Cattle dung	Azolla
Carbon (g/kg)	323.33 ± 1.76	300 ± 2.31
Nitrogen (g/kg)	13.20 ± 0.13	18.56 ± 0.19
C/N	24.50 ± 0.38	16.16 ± 0.25
Phosphorus (g/kg)	5.20 ± 0.11	4.23 ± 0.06
Potassium (g/kg)	11.36 ± 0.27	15.31 ± 0.17
Calcium (g/kg)	6.18 ± 0.04	9.14 ± 0.05
Magnesium (g/kg)	4.87 ± 0.05	5.77 ± 0.04
Copper (g/kg)	15.08 ± 0.05	8.58 ± 0.23
Sodium (g/kg)	1.52 ± 0.02	3.46 ± 0.05
Manganese (mg/kg)	114.53 ± 0.47	321.78 ± 1.64
Zinc (mg/kg)	164.62 ± 1.21	144.50 ± 0.94
Iron (mg/kg)	1979 ± 7.81	4542.33 ± 9.84
Boron (mg/kg)	7.57 ± 0.04	8.78 ± 0.11
Sulfur (mg/kg)	3.14 ± 0.01	3.36 ± 0.05
Hydrogen (mg/kg)	30.84 ± 0.15	32.76 ± 0.19
pH	7.78 ± 0.05	7.82 ± 0.02
EC ($\mu s/cm$)	1.06 ± 0.02	1.54 ± 0.03

Table 1 Initial physico-chemical characteristics of cattle dung and azolla (mean \pm SE)

The experiment was terminated when a mixture was converted to brown earthy material or crumbly balls. The contents of each tub were sieved, dried in an oven for 36 h at 60 °C, packed, and stored separately for chemical analysis. EC and pH of the mixture were estimated with the Decibel soil and water analyzer kit (DB-1202) from distilled water suspension (1:10 w/v). A CHNSO analyzer, Thermo Flash- 2000, was used to measure total organic carbon (TOC), nitrogen (N), hydrogen (H), and sulfur (S). Powdered sample (1–3 mg) was taken in a tin capsule and combusted at 1000 °C with helium as a carrier gas and oxygen for combustion. Elemental analysis was done with the help of Eager experience software. The Thermo Scientific iCAP-6000 series ICP spectrometer was used for estimation of phosphorus (P), potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), boron (B), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn). The sample (0.1 g) was digested in a diacid mixture $(HCl: HNO₃ 1:5)$ in Anton Parr Microwave Multiwave 3000

Table 2 Treatments made by mixing different materials

for 75 min, diluted to 50 ml with double-distilled water, and subjected to iCAP analysis. One-way ANOVA and Tukey's test were used for calculating the variation and significance level ($p < 0.05$) between means of different treatments with the help of SPSS 16 program.

Results and discussion

Bioconversion of the mixtures

A significant difference $(p < 0.01)$ was observed between the days required for bioconversion of the mixtures in the Vcom and Acom groups. Degradation of the mixtures of the Vcom group was observed after 120 to 135 days while degradation in the Acom group was observed after 138–150 days. Vermicompost was dark, brown, granular, and more homogenous in comparison to compost which was lighter in color and lumpy in texture at the time of harvesting. WCA and WCFA were ready for harvesting after 120 days (106 days after pre-composting), WFA and WA were ready in 128 days, WCF was ready in 129 days, and 132 days were required for complete degradation of WC. However, WF and W got decomposed last of all in 135 days. The trend of the rate of degradation of the mixtures of the Acom group was WCA (138 days) < WCFA (139 days) < WFA (142 days) < WA (144 days) < WCF (146 days) < WC (146 days) < WF (148 days) $days)$ < W (150 days). The difference in the rate of degradation of various mixtures can be due to different chemical characteristics of the feed. Variation in palatability, particle size, protein and crude fiber contents, polyphenols, and related substances has been suggested to directly or indirectly influence the decomposing potential of earthworms in treatments (Suthar [2009\)](#page-11-0). Similarly, Velmourougane and Raphael [\(2011\)](#page-11-0) reported that more days were required for composting (205 days) in comparison to vermicomposting (112 days) of coffee pulp by exotic worms. Earthworms play an important role in the decomposition of organic matter and soil metabolism through feeding, fragmentation, aeration, turnover, and dispersion (Ansari and Ismail [2012\)](#page-10-0). In the present study, faster conversion of the mixtures of the Vcom group in

comparison to the mixtures of the Acom group may have been due to fragmentation of the organic matter by earthworms, which might have enhanced microbial degradation due to an increased surface area for the action of microbes. Earthworm gut and its microbial fauna have been reported to cause faster biochemical degradation of organic matter in comparison to microbes alone (Fornes et al. [2012](#page-10-0)). Longer time for degradation in the Acom group of the present study is supported by Mupondi et al. ([2010](#page-11-0)) who reported that during thermophilic composting frequent turning of the material resulted in loss of nutrients and high temperatures $(> 60 °C)$ associated with this process inhibited decomposition.

Earthworm biomass and population buildup

A significant difference $(p < 0.01)$ was observed in earthworm biomass, cocoon, and hatchling production in various mixtures of the present study (Fig. 1). The number of earthworms increased till the 80th day, and a decline was seen after this till the end of the experiment (120th day post earthworm inoculation). On the 80th day, the maximum number of worms was observed in WCA (203 \pm 2.52) followed by WCFA (189.67 \pm 0.88) while it was minimum in W (132.67 \pm 1.45). The trend of increase in the number of worms was WCA (134.67 ± 0.88) $>$ WCFA (125 \pm 1.73) $>$ WCF (117 \pm 0.88) $>$ WC (110 \pm 0.57) $>$ WFA (106.33 \pm 0.88) $>$ WA (102 \pm 1.15) $>$ WF (96.33 \pm) 0.88) > W (91.67 \pm 0.88). Earthworms showed rapid growth in all the mixtures during the initial days, but after the 80th day, growth declined continuously till termination of the experiment; the decline in growth from the 80th to the 120th day could have been due to depletion of nutrients in the mixtures with time.

Malińska et al. ([2016](#page-10-0)), Sharma and Garg [\(2018\)](#page-11-0), and Suthar ([2009](#page-11-0)) also related the decline in earthworm biomass to non-availability of nutrients near the end of vermicomposting of various organic wastes. A similar pattern was seen for worm biomass, where maximum biomass was observed in all the mixtures on the 80th day ranging from 57.85 (W) to 77.04 g (WCA), while on the 120th day, the maximum weight of earthworms was observed in WCA (60.76 ± 0.28) which was followed by WCFA (57.51 ± 0.28) 0.35), WCF (55.67 \pm 0.62), WFA (53.04 \pm 0.08), WC (51.28 ± 0.09) , WA (50.15 ± 0.02) , WF (48.90 ± 0.16) , and W (46.90 \pm 0.45) (Fig. 1). Production of cocoons started during the third to the fourth week in various mixtures and increased till the 80th day and then declined till the end. On the 120th day, the maximum number of cocoons was observed in WCFA (226.33 \pm 5.04) while it was minimum in W (104 \pm 1.15). The trend in cocoon production was WCFA > WCA > $WC > WFA > WCF > WA > WF > W.$ On the other hand, hatchlings were noticed first of all around the 60th day and increased continuously till the end. The maximum (241.67 \pm 3.84) and minimum (102 ± 1.15) numbers of hatchlings were observed in WCA and W, respectively, on the 120th day. The trend of increase in the number of hatchlings was WCA > $WCFA>WC > WFA > WCF > WA > WF > W.$

Fig. 1 Population buildup of E. fetida in various mixtures of wheat straw, azolla, cattle dung, and fungus. *Values with different superscripts are significantly different $(p < 0.01)$

Results of the present study clearly show that although WCA supported maximum growth of worms, WCFAwas best for overall growth and reproduction of E. fetida. It seems that azolla, cattle dung, and A. terreus enhanced the palatability of wheat straw/stubble for E. fetida and provided it assimilable energy that was used by the worm for growth and reproduction. An increase in cocoon production of Perionyx excavates during vermicomposting of agricultural residues, farmyard manure, and cattle dung was related by Suthar ([2007](#page-11-0)) to the biomass and activities of microbes as well as more availability of nutrients. The difference in cocoon production in the present study could also be attributed to the quality of initial feed mixtures (Table [3\)](#page-5-0) as suggested by Edwards et al. ([1998](#page-10-0)). The presence of cow and goat dung in the mixtures of agro-wastes (wheat straw/stubble, banana peels) and different brans (barley, gram, and rice) has been suggested to enhance earthworm maturation and production of cocoons and hatchlings (Garg [2005;](#page-10-0) Chauhan and Singh [2013](#page-10-0)) which directly supports the observed higher growth and reproduction of E. fetida in the mixtures having cattle dung in the present study. Azolla and fungus were observed to have a costimulatory effect on growth and reproduction of E. fetida in various mixtures of wheat straw/stubble.

Physico-chemical characteristics

A significant difference $(p < 0.01)$ was observed in physicochemical characteristics of various mixtures, and a greater increase was observed in the products of the Vcom group in comparison to the Acom group (Tables [4](#page-6-0) and [5\)](#page-7-0). A significant decrease over the control $(p < 0.01)$ was observed in the content of total organic carbon (TOC), C/N ratio, pH, and EC while an increase over the control was observed in N, P, K, H, S, Na, Ca, Mg, Cu, B, Mn, Zn, and Fe of all the mixtures of both Vcom and Acom groups. WCA (21.74%) showed maximum reduction over control in TOC which was followed by WCFA (18.25%) while it was minimum in WF (2.50%) in the Vcom group. On the other hand, a decline in carbon content over that of the control ranged from 3.53% (WC) to 16.14% (WCA) in the Acom group. The general trend of decrease was $WCA > WCPA > WA > WFA > WCF > WC > WF > W$ in the Vcom group and $WCA > WA > WCPA > WFA > WCF > WF$ > WC > W in the Acom group. Higher decline in TOC of the Vcom group in the present study may have been due to loss of organic C as labile forms (carbon dioxide/carbon monoxide/ $CH₃$) and its use as an energy source by earthworms and microorganisms (Bhattacharya and Chattopadhyay [2004](#page-10-0)). Higher decrease in TOC during vermicomposting of crop residues has been attributed to higher loss as carbon dioxide due to combined respiration of microbes and earthworms (Suthar [2007,](#page-11-0) [2009\)](#page-11-0). The report of Suthar et al. ([2014](#page-11-0)) that mutualism between earthworms and their gut microbes intensifies the mineralization rate of carbon in the feed mixtures during

vermicomposting and results in higher decline in TOC in the end product directly supports the observed much lower decline in TOC of the Acom group compared to the Vcom group.

In the Vcom group, increase over control in the content of nitrogen in WCA (32.46%) was > WCFA (23.64%) > WA (15.88%) > WFA (15.53%) > WCF (14.49%) > WC (9.57%) > WF (4.21%) , while the trend of increase over control in the Acom group $[1.36\%$ (WF)–27.89% (WCA)] was WCA > WCFA>WFA > WA > WCF > WC > WF > W. All the mixtures with azolla showed a greater increase in nitrogen (WCA, WCFA, WA, and WFA). This may be due to the initial higher nitrogen content of azolla (18.2 g/kg, Table [2\)](#page-2-0). The improved C/N ratio of the mixtures therefore not only led to faster bioconversion but also resulted in higher N of the products. A. azollae (symbiotic cyanobacterium) fixes atmospheric nitrogen (Raja et al. [2012](#page-11-0)), and addition of azolla has been reported to improve the structure and chemical composition of soil (Hakeem et al. [2016](#page-10-0); Subedi and Shrestha [2015](#page-11-0)). Mineralization of organic matter during vermicomposting may have been responsible for higher content of nitrogen in the products of the Vcom group (Viel et al. [1987;](#page-11-0) Das et al. [2014;](#page-10-0) Shak et al. [2014\)](#page-11-0). Release of nitrogenous products with excreta, urine, mucoproteins, growth-stimulating hormones, and enzymes by earthworms and their addition after decomposition of dead decaying tissues of worms may have collectively caused a higher increase in N content of the mixtures of the Vcom group as suggested by Pattnaik and Reddy [\(2010\)](#page-11-0), Tripathi and Bhardwaj [\(2004\)](#page-11-0), Das et al. [\(2017\)](#page-10-0), Suthar [\(2007\)](#page-11-0), and Vig et al. [\(2011](#page-11-0)).

Previous studies have also reported a greater decline in the C/N ratio of Vcom (Kaur et al. [2010;](#page-10-0) Mistry et al. [2015;](#page-11-0) Arora et al. [2019](#page-10-0)). Reduction over control in the C/N ratio of WCA $(40.89\%, 34.45\%)$ was > WCFA $(33.86\%, 29.40\%)$ > WA $(25.71\%, 21.95\%)$ > WFA $(25.50\%, 21.95\%)$ > WCF $(19.66\%, 18.84\%)$ > WC $(15.76\%, 11.41\%)$ > WF $(6.45\%,$ 8.63%) of the Vcom and Acom groups respectively. Azolla is commonly used as a biofertilizer as it supplies fixed nitrogen to the soil (Yadav et al. [2014](#page-11-0)). This might be the reason for the higher reduction in the C/N ratio of the mixtures with azolla (WCA, WCFA, WA, and WFA) in both the groups in the present study. It is well known that plants cannot assimilate nitrogen unless the C/N ratio is below 20 (Senesi [1989;](#page-11-0) Edwards and Bohlen [1996](#page-10-0)); therefore, it is very clear from the data that the addition of azolla improves the fertilizer value of vermicomposted wheat straw/stubble for the crops. The decrease in the C/N ratio indicates increased humification of organic matter (Pigatin et al. [2016](#page-11-0)) as well as maturity of the compost and vermicompost (Ravindran et al. [2015](#page-11-0), [2016](#page-11-0)); therefore, vermicomposting of wheat straw/stubble will yield a better product.

The trend of increase over control in P was WCA (101.82%) > WCFA (100.87%) > WCF (85.96%) > WFA

Treatments Parameters

Treatments

Parameters $Ca(g/kg)$

 $\text{Ca}(g/\text{kg})$ Mg (g/kg) Cu (mg/kg) B (mg/kg) B (mg/kg) Fe (mg/kg) Zn (mg/kg) Mn (mg/kg) pH

 $B(mg/kg)$

Fe (mg/kg)

 $W=0.04^f=0.04^f=0.04e+0.04e+0.02^f=0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e+0.05e$ WC 3.13 \pm 0.04ef 1.23 \pm 0.17e 10.28 \pm 0.03c 20.90 \pm 0.03c 1543.17 \pm 13.52c 52.31 \pm 0.02f 40.23 \pm 0.03c WA 4.39 ± 0.03c 1.54 ± 0.043d 1.12 ± 0.04b 21.20 ± 0.03d 10.51d 53.45 ± 0.05d 53.45 ± 0.05d 43.96 ± 0.07 ± 0.01d $\text{WF} = 2.28 \pm 0.06^\text{g} = 1.18 \pm 0.01^\text{e} = 8.90 \pm 0.02^\text{e} = 19.31 \pm 0.02^\text{e} = 17.5 \pm 7.15^\text{f} = 0.25^\text{g} = 0$ WCF 3.16 \pm 0.01^e 2.15 \pm 0.03^c 10.15 \pm 0.03^c 1729.50.5c^d 54.27 \pm 0.02^c 54.21 \pm 0.02^e 54.51 ± 0.02^e $\rm{WCA} = 5.30 \pm 0.05^a \ \rm{Z} - 5.7 \pm 0.04^b \ \rm{11.18 \pm 0.05^b} \ \rm{23.34 \pm 0.07^b} \ \rm{37.83 \pm 7.59^a} \ \rm{57.87 \pm 2.03^a} \ \rm{57.83 \pm 2.59 \pm 0.03^b} \ \rm{57.83 \pm 2.03^b} \ \rm{57.83 \pm 2.03^b} \ \rm{57.83 \pm 2.03^b} \ \rm{57.83 \pm 2.03^b} \$ WFA 3.53 ± 0.03^d 1.25 ± 0.01° 9.73 ± 0.07d 1.250^d 1784.33 ± 4.10^c 52.50 ± 0.01e 48.75 ± 0.04° 1.56 ± 0.01° $\rm{WCFA} \ \rm{4.85\pm0.04} \ \rm{2.99\pm0.02} \ \rm{11.71\pm0.07} \ \rm{11.71\pm0.07} \ \rm{13.4\pm0.07} \ \rm{5.33\pm6.21} \ \rm{5.34\pm0.28} \ \rm{5.63\pm0.02} \ \rm{14.71\pm0.02} \ \rm{17.71\pm0.02} \ \rm{18.73\pm0.02} \ \rm{18.73\pm0.02} \ \rm{19.73\pm0.02} \ \rm{19.73\pm$

 $18.75 \pm 0.05^{\circ}$ 20.90 ± 0.03 ^d

 1075.33 ± 15.94^8

 8.27 ± 0.01^b 7.97 ± 0.01^d

> 37.75 ± 0.25^8 47.62 ± 0.12^d $50.50 \pm 0.09^{\rm a}$

 43.96 ± 0.17^e

 $53.45 \pm 0.05^{\rm d}$ 51.38 ± 0.02 ^g

 1704.33 ± 10.51 ^d $1543.17 \pm 13.52^{\circ}$

 21.20 ± 0.03 ^d

 11.12 ± 0.04^b

 1.54 ± 0.03 ^d 1.18 ± 0.01 ^e 2.15 ± 0.03 ^c

 $8.90 \pm 0.02^{\circ}$

 10.28 ± 0.03 ^c

 $8.58\pm0.02^{\rm f}$ Cu (mg/kg)

 0.04°

 $Mg(g/kg)$

 1.23 ± 0.17^e

 3.13 ± 0.04 ^{ef} $2.96\pm0.04^{\mathrm{f}}$

> WC $\mathbb{W}\!A$

 \geq

 $4.39 \pm 0.09^{\circ}$

 2.28 ± 0.06^8 $3.16 \pm 0.01^{\circ}$

> WCF **WCA** WFA

 W_{F}

 $19.31 \pm 0.02^{\circ}$ 21.88 ± 0.03 ^c 23.34 ± 0.07^b

 11.18 ± 0.05^b

 2.67 ± 0.04^b

 $1.25 \pm 0.01^{\circ}$ 2.99 ± 0.02^a

 3.53 ± 0.03^d 4.85 ± 0.04^{b}

WCFA

 5.30 ± 0.05^a

 $11.71\pm0.07^{\mathrm{a}}$

 9.73 ± 0.07^d

 $10.15 \pm 0.02^{\circ}$

 $20.70 \pm 0.50^{\rm d}$ 24.10 ± 0.03 ^a

 1237.67 ± 7.15^f 729.50 ± 7.20^d 971.83 ± 7.59^a $784.33 \pm 4.10^{\circ}$ 875.33 ± 6.21^b

 52.31 ± 0.02^f

 $40.23 \pm 0.04^{\text{f}}$

 8.46 ± 0.01^{a} $8.15 \pm 0.03^{\circ}$

 $36.31\pm0.08^{\mathrm{h}}$

 $50.58 \pm 0.05^{\rm h}$

 Zn (mg/kg)

Mn (mg/kg)

 $E_{\rm g}$

 $7.39\pm0.03^{\rm h}$ $7.56 \pm 0.01^{\circ}$ 7.47 ± 0.02 ^g

 49.70 ± 0.06^b

 $48.75 \pm 0.04^{\circ}$

 $52.50 \pm 0.01^{\circ}$ 56.30 ± 0.02^b

 $54.27 \pm 0.02^{\circ}$ 57.87 ± 0.03 ^a

 7.73 ± 0.02^e

Values with different superscripts are statistically ($p < 0.01$) different Values with different superscripts are statistically $(p < 0.01)$ different

Table 4 Final physico-chemical characteristics of various mixtures of wheat straw of Vcom and Acom groups Table 4 Final physico-chemical characteristics of various mixtures of wheat straw of Vcom and Acom groups

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Values in parentheses are percent change over control. Values with different superscripts are statistically $(p < 0.01)$ different

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Table 5 Final physico-chemical characteristics of various mixtures of wheat straw of Vcom and Acom groups Table 5 Final physico-chemical characteristics of various mixtures of wheat straw of Vcom and Acom groups

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Values in parentheses are percent change over control. Values with different superscripts are statistically $(p < 0.01)$ different

Values in parentheses are percent change over control. Values with different superscripts are statistically $(p < 0.01)$ different

 $(80.50\%) > \text{WC } (64.99\%) > \text{WA } (49.54\%) > \text{WF } (11.53\%)$ in Vcom group. However, the increase over control in P in the Acom group ranged from 6.87% (WF) to 95.17% (WCA) and the trend was $WCA > WCFA > WCF > WFA > WA > WC >$ $WF > W$. Maximum increase over control in the content of K in both Vcom and Acom groups was seen in WCA (27.69% and 25.58% respectively) while it was minimum in WF (3.34% and 2.73% respectively). The trend of increase was, however, slightly different in the Vcom (WCA > WCFA > $WA > WCF > WC > WFA > WF > W$) and the Acom group $(WCA > WCFA > WA > WC > WCF > WFA > WF > W).$ Other authors (Suthar [2007;](#page-11-0) Kaur et al. [2010](#page-10-0)) have also reported a higher increase in P with vermicomposting in comparison to aerobic composting. More increase can also be attributed to net higher loss of dry matter leading to concentration of phosphorus in the final products of Vcom group (Bhat et al. [2018](#page-10-0); Bhat et al. [2017](#page-10-0)). Increase in phosphate in the vermicompost occurs due to the production of a considerable amount of alkaline phosphatases in the worm gut that enriches the vermicast with phosphorus (Lakshmi et al. [2013](#page-10-0); Das et al. [2014\)](#page-10-0). An increase in phosphorus in the vermicompost over initial feed materials (decanter cake and rice straw) has been reported by Lim et al. ([2016](#page-10-0)). Higher P and K in the vermicomposts have been attributed to the reduction in weight and degradation of labile organic compounds through the release of $CO₂$ (Malińska et al. [2016\)](#page-10-0). Lesser increase in phosphorous in the products of the Acom group indicates that the activity of earthworms in addition to microbial activity during vermicomposting reduces immobilization of phosphorous (Kaur et al. [2010](#page-10-0)) in the products of the Vcom group.

An increase over control in hydrogen in the Vcom and Acom groups was maximum in WCA (5.10 and 4.88%, respectively) and minimum in WF (1.03 and 0.97%, respectively). The trend of increase over the control in the Vcom and Acom groups was $WCA > WCPA > WCF > WFA > WA > WC > WF > W$ and $WCA > WCF > WCPA > WFA > WA > WC > WF > W$, respectively. Hydrogen (an essential element) in the soil exists in various forms (organic and inorganic) and is used by plants during photosynthesis. It forms bonds with different elements like nitrogen, oxygen, and carbon and is exchangeable with water and water vapors (Paul et al. [2016\)](#page-11-0). In the present study, increase in hydrogen in both the groups (Acom and Vcom) may have been due to mineralization of organic matter. Similarly for S also, the increase over control was maximum in WCFA (40.18 and 38.22%) and minimum in WF (4.82 and 6.62%) in the Vcom and the Acom group, respectively. The trend of increase in sulfur was WCFA > WCA > WFA > WCF > WA > WC > WF > W for the Vcom group and WCFA $>$ WCA $>$ WFA $>$ WCF $>$ WC $>$ $WA > WF > W$ for the Acom group. Sulfur is an important key element required by plants for the activity of different enzymes and vitamins and the formation of chlorophyll. A higher increase

in sulfur in the Vcom group can be attributed to the joint action of earthworms and microbes as suggested by Das et al. [\(2012\)](#page-10-0).

Maximum decline over control in EC was seen for WCA (10.54%, 13.92%), and it was minimum in WF (3.82%, 4.19%) for the Vcom and Acom group, respectively. Maximum and minimum decline over control in pH was also observed in WCA (12.55% and 11.75%) and WF (1.42% and 1.21%) in the Vcom and Acom group, respectively. The trend of decline over control for EC (WCA > WCFA > WFA > $WCF > WA > WF > WC > WCA$) was the same for both the groups while the trend of decrease over the control in pH was WCA > WCFA > WCF > WFA > WA > WC > WF > W for the Vcom group and $WCA > WCPA > WFA > WCF >$ $WA > WC > WF > W$ for the Acom group. EC is a measure of the amount of salinity in the material and is a good indicator of vermicompost quality and/whether it can be used in agriculture or not. In the present study, the percent decrease over control in EC was more in the Acom group in comparison to the Vcom group. The decrease in EC during vermicomposting of rice straw and husk was suggested by Shak et al. [\(2014\)](#page-11-0) to be due to production of soluble metabolites and precipitation of dissolved salts. The decrease in the EC could also be attributed to the precipitation of mineral salts as reported by Lim et al. ([2012](#page-10-0)). Higher EC of the final product can inhibit plant rooting and reduce the transportation of soil nutrients to plants (Singh and Kalamdhad [2016\)](#page-11-0). However, an increase in EC of the vermicompost has been reported by various authors; they suggested that an increase in the level of soluble salts due to mineralization of organic matter was responsible for the increase (Kaur et al. [2010;](#page-10-0) Das et al. [2017\)](#page-10-0). More reduction in pH of the products of the Vcom group in the present study may be due to higher loss of carbon and greater production of organic acids by the joint action of microbes and earthworms as reported by Bhat et al. ([2015](#page-10-0), [2017\)](#page-10-0) and Garg et al. [\(2006\)](#page-10-0). The accumulation of organic acids in the Vcom has been suggested to be due to the action of acidogenic microbes of the earthworm gut (Elvira et al. [1998\)](#page-10-0). At the same time, fulvic and humic acids (rich in Vcom) have also been reported to be responsible for a decline in pH of the vermicompost (Ndegwa and Thompson [2000\)](#page-11-0).

Increase over control in the content of Na was 7.21% (W)– 86.29% (WCA) in the Vcom group and 4.42% (W)–81.25% (WCA) in the Acom group. The trend of increase in Na was $WCA > WCPA > WFA > WA > WC > WCF > WF > W$ for the Vcom group and WCA $>$ WCFA $>$ WFA $>$ WA $>$ WC $>$ WCF > WF > W for the Acom group. The trend of increase over control in the content of Mg was in the order of WCA (43.39%) > WCFA (39.93%) > WCF (36.50%) > WFA $(29.97\%) > WA (25.24\%) > WC (17.95\%) > WF (2.96\%)$ for the Vcom group, whereas for the Acom group, the trend was WCA (40.37%) > WCFA (30.75%) > WCF (30.40%) > WA (18.87%) > WFA (15.56%) > WC (9.44%) > WF (1.64%). In the Vcom group, percent increase over control

in Ca ranged from 24.07% (WCA) to 2.67% (WF) while in the Acom group, it ranged from 1.77% (WF) to 19.56% (WCA). The trend of increase was the same (WCA > WCFA > WCF > $WA > WFA > WC > WF > W$) for both Vcom and Acom groups. Higher content of Na, Mg, and Ca in the products of the mixtures having azolla (WCA, WCFA, WFA, and WA) in both the groups could have been due to higher content of these nutrients in azolla (Table [1\)](#page-2-0). Anitha et al. [\(2016\)](#page-10-0) also reported high content of calcium (1.64%), potassium (2.71%), and phosphorus (0.34%) in azolla. However, the net loss of dry mass in the final products may also be responsible for the increase in nutrients of the products (Bansal and Kapoor [2000;](#page-10-0) Suthar [2009](#page-11-0)). The gut microflora of earthworms and mucus secreted by earthworms have been reported to accelerate degradation and mineralization of organic matter and have been suggested to enrich the final product (vermicompost) with more nutrients (Singh et al. [2014](#page-11-0)).

Suthar [\(2010](#page-11-0)) related the higher content of magnesium in the final products of the vermicompost to the colonization of fungal and microalgal hyphae in the fresh cast of earthworms. The increase in calcium content of vermicompost can be attributed to metabolism of calcium in the calciferous glands of earthworms and release of excess calcium as bicarbonate in the casts (Spiers et al. [1986](#page-11-0); Shak et al. [2014\)](#page-11-0). Ions like Ca^{2+} , Mg^{2+} , and K^+ are essential for plant growth (Singh and Kalamdhad [2016](#page-11-0)); therefore, a significant increase in the concentration of these ions in the products of WCA, WCFA, and W indicates the plant growth-promoting value of these products.

Increase in the concentration of boron ranged from 2.89% (W) to 12.91% (WCFA) in the products of the Vcom group, while it ranged from 1.80% (W) to 11.97% (WCA) in the products of the Acom group. The trend of increase for boron in the Vcom group was WCFA > WCA > WCF > WFA > WA > WC > WF > W, and for the Acom group, it was WCA > WCFA > $WFA > WCF > WA > WC > WF > W$. Increase over the control for copper in WCFA (32.81%, 31.07) was > WCA (31.07%, 27.97%) > WFA (22.78%, 17.51%) > WCF (20.48%, 16.77%) $>$ WA (19.49%, 15.47%) $>$ WC (16.36%, 8.80%) $>$ WF (2.53%, 1.65%) in the Vcom and Acom group, respectively. Increase over control in Mn ranged from 3.80% (WF) to 13.96% (WCA) in the Vcom group whereas it ranged from 2.99% (WF) to 12.37% (WCA) in the Acom group. The trend of increase in Mn for the Vcom group was WCA > WCFA > WFA > $WA > WCF > WC > WF > W$, and for Acom group, it was WCA > WA > WCFA > WFA > WCF > WC > WF > W. Both Zn and Fe were more in the Acom group in comparison to the Vcom group. Increase over control in zinc ranged from 0.65% (WF) to 5.79% (WCA) in the Vcom group and from 0.80% (WF) to 7.07% (WCA) in the Acom group. The trend of increase in the Vcom and the Acom groups was WCA > WCFA > WFA > WA > WCF > WC > WF > W and WCA > WCFA>WFA > $WCF > WA > WC > WF > W$, respectively. In the Vcom group, there was 3.56% (WF)–69.29% (WCA) increase over control in

the content of Fe whereas in the Acom group, increase over control ranged from 4.86% (WF) to 71.88% (WCA). The trend of increase was WCA > WCFA > WFA > WA > WCF > WC > WF > W for both the groups. The mixtures WCA and WCFA had higher concentration of micronutrients compared to other mixtures. Previous studies have reported that the increase in metal (Fe, Cu, and Zn) concentration after vermicomposting of organic wastes was due to reduction of the weight and volume of substrates during the breakdown of organic matter (Yadav and Garg [2011;](#page-11-0) Shak et al. [2014;](#page-11-0) Pigatin et al. [2016\)](#page-11-0) or due to mineralization of feedstock (Sharma and Garg [2018\)](#page-11-0). However, Taguiling [\(2016](#page-11-0)) reported an increase in the content of Zn and Fe but a decline in the contents of Cu and Mn after vermicomposting rice straw and cornstalks mixed with green biomass (Leucaena leucocephala, Tithonia diversifolia, and Moringa oleifera) in different ratios.

Conclusion

Wheat straw/stubble, a recalcitrant bioresource, can be converted into a nutrient-rich product in 120 days, when it is amended with azolla, A. terreus, and cattle dung [666 g (W) + 294 g (C) + 20 g (A) + 20 ml (F)] and subjected to vermicomposting with E. fetida. Degradation of WCA [666 g (W) + 314 g (C) + 20 g (A)] was also observed after 120 days, at the same time quality of its end product was better. Higher nutrient content along with low C/N (14.83) ratio and EC (1.13) indicates that the product of this mixture is good for plant growth. Therefore, azolla not only helps in faster recycling of nutrients from wheat straw/stubble but also reduces the amount of cattle dung required for its bioconversion. Vermicomposting with Azolla pinnata can be adopted as an alternative to burning for ecosafe disposal of surplus wheat straw/stubble for future agronomic use.

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