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BIOCHEMICAL COMPOSITION OF ENRICHED INSECT BIOMASS COMPOST AT DIFFERENT STAGES OF COMPOST PREPARATION

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ABSTRACT

An experiment was conducted at the College of Sericulture, Chintamani, Chickkaballapur District, Karnataka, India to study the influence of compost enrichment with selected insect species and its biochemical composition at different stages of compost preparation. The amounts of humic acid produced by all enriched compost treatments were more than the amounts of fulvic acid produced. At 90 days, higher humic acid and fulvic acid content was found in treatment (C2 - FYM + silkworm pupae). The levels humic acid index was noticed to be higher in the silkworm pupae enriched compost followed by moth enriched compost, E_4/E_6 is a measure of aromaticity and it showed lower values in (C1) treatment which received, farmyard manure and cow dung. Higher E_4/E_6 values were observed in treatment C2 silkworm pupae compost. Fulvic acid had a higher total acidity, phenolic group, and carboxylic group when compared to humic acid. Carboxylic group was highest in C2 (FYM + silkworm pupae) of 4.50 meq g^{-1}. The total acidity of fulvic acid was highest in C2 (FYM + silkworm pupae) of 6.66 meq g^{-1} followed by C3 (FYM + silkworm moth).

***Key words*: Humic acid; Fulvic acid; Carboxylic group; Total acidity; Phenolic group.**

Introduction

Currently, everyone around the world must simultaneously address the problems of feeding a growing population, conserving the environment, and developing sustainable energy sources. By 2030, there will be a 2-5 times increase in food demand, and in order to meet this demand, food production is estimated to rise by 60% (Clair and Lynch, 2010). Inputs made from non-renewable energy sources, such as synthetic fertilisers, propelled agricultural intensification during the 20th century. Despite the fact that these methods considerably increased crop yields, they have also contributed to a significant loss of ecological legacy due to deforestation, soil erosion, industrial pollution, reductions in surface and groundwater quality, and loss of biodiversity (including genetic erosion). There is no hint of a slowing down in the frightening rate at which these detrimental effects of food production are occurring (Altieri, 2002).

Humic substances (HS) are the primary source of organic carbon at the surface of the planet and are produced through the chemical and biological alteration of plant and animal materials as well as microbial metabolism. They assist in the control of numerous important ecological and environmental processes. For example, Plant growth and terrestrial life in general are sustained by HS, which also controls the cycling of soil carbon and nitrogen, the development of plants and microbes, the fate and movement of substances produced from humans and heavy metals, and the stabilisation of soil structure. In the past ten years, the discovery that humus is a self-assembled supramolecular association of tiny heterogeneous molecules held together mostly by weak hydrophobic connections has made significant strides in our understanding of humus chemistry (Piccolo, 2002). In solution, HS is better represented as a variety of relatively low-molecular-mass components that establish dynamic associations and are supported by hydrogen bonds and hydrophobic interactions. The hydrophilic/hydrophobic ratio controls how reactive it is to the environment (Piccolo, 2012).

The operationally-defined fractionation of humic compounds, which was initially proposed by Sprengel in 1837, is based on their solubility, according to Hayes, (2006). Humic acids (HA) are described by soil scientists as humus components that are soluble in aqueous alkaline solutions but precipitate when the pH is raised to 1-2. In contrast, after the aqueous alkaline extracts are acidified, fulvic acids (FA) remain in solution. This traditional term is still used in older scientific publications, although technically speaking, HS are nothing more than the end result of an alkaline extraction process from soils and sediments that produces a saponification reaction. FA were redefined by Piccolo, (2002) as associations of small hydrophilic molecules with sufficient acid functional groups to maintain the fulvic clusters dispersed in solution at any pH, as opposed to humic acids, which are associations of predominantly hydrophobic compounds (polymethylenic chains, fatty acids, steroids compounds) stabilised at neutral pH by hydrophobic dispersive forces. As intermolecular hydrogen bonds form more frequently at lower pH levels and humic matter flocculates, their conformations gradually increase in size.

Insects play a crucial role in many ecosystems, as they carry out key tasks like pollinating flowers, aerating the soil, and controlling pests and other insects. Numerous insects, particularly beetles, are scavengers that consume dead animals and fallen trees in order to replenish the environment's nutrients. Insects act as decomposers, assisting in the formation of the topsoil's nutrient-rich layer. Burrowing insects such as beetles and ants create tunnels that serve as water routes for plants, which are profitable. In addition, insects enrich the soil by excreting a range of nutrients in their droppings. In various regions of the world, people ingest insects as food. In many third-world countries, insects are considered as delicacy because they are a valuable source of protein, vitamins, and minerals. It is extremely rare to locate one bug that humans do not consume in some way. The most well-liked insects include cicadas, locusts, mantises, grubs, caterpillars, crickets, ants, and wasps. The idea of including a source of protein in human nutrition is backed by a lot of people. From South America to Japan, people eat roasted insects like grasshoppers or beetles (Huis *et al*., 2013). Because of the high nutrient content of insects, we planned to incorporate these insect species in the compost with the aim of enriching the compost. The main objective is to study the biochemical composition of enriched insect biomass compost at different stages of compost preparation

Material and Methods

The composts in the field were prepared at the College of Sericulture, Chintamani. The experiment was done during the period from November 2020 to February 2021 (90 days).

Materials used in composting

The main raw materials used in the preparation of composts were FYM (farm yard manure), silkworm pupae, silkworm moth, uzi fly and fruit fly. Silkworm pupae were obtained from Alamagiri village reeling unit Chintamani, Chickkaballapur district. Silkworm moths were collected from Govt. Sericulture grainage unit Chintamani, Chickkaballapur district (CSB) . Uzi flies were collected from cocoon markets Sidlagatta, Chintamani and Chickkaballapur and fruit flies were collected from mass trapping method by using cue-lure traps which attract male fruit flies in cabbage, tomato and mango orchards. Farm yard manure and Cow dung was obtained from the Agricultural Research Station (ARS), Chintamani. The slurry was prepared by adding 100 g of cow dung/ 1 liter of water.

An aerobic method of compost was prepared. The basic raw materials used for composting were raw silkworm pupae, silkworm moth, uzi fly and fruit fly. The organic additives used were farm yard manure and cow dung slurry. Compost was prepared in compost pits of size 7m × 4m × 3m (length × breadth × height).

Requirements for insect biomass compost preparation

Raw insect species waste: 05 kg

Farm Yard Manure: 20 kg

Cow dung slurry @10% w/w

Treatments used for preparation of bio composts:

C1- Control- (FYM) + Cow dung slurry

C2- Silkworm pupae + FYM + Cow dung slurry

C3- Silkworm moth + FYM + Cow dung slurry

C4- Uzi fly + FYM + Cow dung slurry

C5- Fruit fly+ FYM + Cow dung slurry

(Insect biomass and FYM mixed in the ratio of 1:4 N equivalent ratios).

It was replicated in four pits, and the composting process lasted three months. Every 30 days, the decomposing materials were turned. Turnings were provided during the composting process. By the 90th day, the compost was complete.

Sample collection

As part of the monthly turning, samples were gathered from each compost pit using spiked augers and pooled, and composite samples formed and analysed. The samples were dried in the shade and analysed for biochemical characteristics. Extraction, fractionation, and quantification of humic substances by following standard procedures described below.

Table: Methodology of different parameters.

Parameter	Method	Referance	Procedure
Total acidity	Ba(OH)2 method	Schnitzer and Gupta (1964).	The sample was allowed to react with an excess of Ba(OH)2. The unreacted Ba(OH)2 was determined by back titrating with standard acid
Carboxyl groups	0.1 N NaOH	Schnitzer and Khan, 1972	liberation of acetic acid when acids are treated with calcium acetate and its titration was carried out with standard 0.1 N NaOH
Phenolic –OH groups	By calculation	——	the difference between total acidity and –COOH acidity.
E_4/E_6 ratio	Spectrophotometer	——	A known quantity of the sample was taken and dissolved in 10 ml of 1×10^2 M $NaHCO_3$ solution

Characteristics of humic fractions from insect biomass compost

Humic substances from insect biomass compost were extracted, fractionated, purified, and characterized using the standard procedures as described below.

Extraction of humic substances

Ten grams of air-dried insect biomass compost sample was weighed into a 250 mL conical flask 100 mL of 0.1 N NaOH was added (Schnitzer and Skinner, 1968) and shaken for 24 hours. The dark-colored supernatant solution was separated by centrifugation and collected. The extraction procedure was repeated thrice using 50 mL of extractant each time for complete extraction of the humic substances.

Fractionation and purification of humic substances

Centrifugation was used to separate the precipitated humic acid fraction. As stated by Stevenson, (1981), precipitation and centrifugation were repeated to achieve partial purification of the humic acid component. The fractions were further refined by treating them for 24 hours with an HCL-HF mixture (5 mL of each HCL and HF acid dissolved in 990 mL of double distilled water), and this acid mixture was separated by centrifugation. To get humic acid, the residue was carefully washed with distilled water and freeze-dried.

Characterization of humic acid

Purified samples of humic acid extracted from different organic wastes were subjected to total acidity, Carboxyl groups, Phenolic groups, E_4/E_6 ratio and Humic acid index.

Results and Discussions

Percent recovery of humic acid and fulvic acid at different stages of enriched insect biomass compost.

The results of the recovery percentage of humic acid and fulvic acid from insect biomass compost at 30, 60 and 90 days after composting are presented in Table 1, Fig. 2. Compost treated with different treatments differed significantly in humic acid and fulvic acid content. Higher recovery of 4.94, 5.94, 7.52 and 2.80, 3.30, 3.90 % humic and fulvic acid at 30, 60 and 90 days after composting was recorded for farm yard manure + silkworm pupae (C2) enriched compost followed by (C3) farm yard manure + silkworm moth and farmyard manure + fruit fly (C5) treatments. The results are in agreement with the work carried out by Satisha and Devarajan *et al.*, (2011) and Gayathri *et al.*, (2011). However lower humic and fulvic acid 4.15, 4.34, 5.06 % of humic acid in farm yard manure treatment (C1) and 0.50, 4.10, 2.20 % fulvic acid was recorded in farm yard manure + fruit fly (C5) treatment. Scheild *et al.*, (1989) reported that, in sewage sludge

Table 1: Total acidity of humic acid and fulvic acid extracted from different enriched insect biomass during different stages of compost.

Insect biomass compost	Total acidity (meq g^{-1})					
	Humic acid			Fulvic acid		
	30 days	60 days	90 days	30 days	60 days	90 days
C1	1.80	1.91	1.98	2.50	3.47	3.99
***C2**	2.87 (59.44)	3.08 (61.26)	3.27 (65.15)	4.50 (80.00)	4.80 (38.33)	6.66 (66.92)
***C3**	2.40 (33.33)	2.66 (39.27)	2.87 (44.95)	4.18 (67.20)	4.68 (34.87)	5.90 (47.87)
***C4**	2.00 (11.11)	2.55 (33.51)	2.78 (40.40)	3.40 (36.00)	4.55 (31.12)	4.85 (21.55)
***C5**	3.87 (115.0)	2.25 (17.80)	3.01 (52.02)	3.55 (42.00)	4.17 (20.17)	5.10 (27.82)
SEm ±	**0.034**	**0.018**	**0.020**	**0.032**	**0.022**	**0.042**
CD at 5%	**0.10**	**0.06**	**0.06**	**0.10**	**0.07**	**0.13**

* Insect biomass and FYM mixed in the ratio of 1:4 N equivalent ratios
The figures in parenthesis indicate percent increase and decrease (+/-) in moisture content (%) over farm yard manure (FYM)

materials, low humic acid recovery is observed.

Due to its elemental makeup and melanin pigment, humic acid derived from organic waste has a tendency to be dark brown in colour (Kumuda, 1987). Farmyard manure treatment (C1) has the least humic acid since no additional insect biomass was added to the mixture. So it is clear that humification was occurring and that further decomposition needed some time. As a result, it might be inferred that humification has not yet been fully finished and that more decomposition is necessary. Treatment C2 had the greatest humic and fulvic acid values, suggesting that this approach may be the most effective for humification. Increasing quantities of humic acid, according to Chefetz *et al*., (1996), indicate the compost's level of humification and maturity. The biological makeup of the raw material has an impact on the humification process.

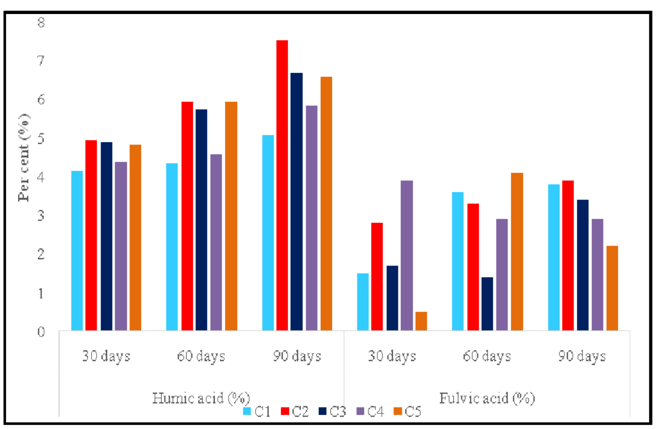

Fig. 1: Changes in humic acid and fulvic acid during different stages of enriched insect biomass compost.

Functional groups of humic acid and fulvic acid extracted from different enriched insect biomass during different stages of composting

Carboxyl groups

Carboxyl group contents of humic acid was ranged from 0.76, 1.26 and 1.46 meq g^{-1} in C1 to 1.76, 2.16 and 2.56 meq g^{-1} in C2 (farmyard manure + silkworm pupae) were observed at 30, 60 and 90 days after composting (Fig. 2). A similar trend was observed with fulvic acid it ranged from 1.20, 2.60 and 3.00 meq g^{-1} in C1 (farmyard manure) and 2.80, 3.20 and 4.50 meq g^{-1} was observed in C2 at 30, 60 and 90 days after composting. Higher content of carboxyl groups in fulvic acid 4.50 meq g^{-1} in C2 at 90 days after composting may be a result of Humic acid degradation is followed by polymerization or condensation; the particle weight is low due to decarboxylation, which is associated with a high molecular weight, indicating humic acid degradation, which may also result in a high amount of carboxyl groups (Lal and Mishra, 2000, Srilatha *et al*., 2013).

Phenolic groups

The phenolic -OH group contents of humic acid ranged from 0.52 meq g^{-1} (C1) to 1.12 meq g^{-1} (C5) at the 90th day after composting while it was 0.99 (C1) to 2.50 meq g^{-1} (C3) in fulvic acid (Fig. 3). A detailed examination of the data on functional groups indicated that humic and fulvic acids had more carboxyl content than phenolic -OH groups, indicating that the carbohydrates and phenolic compounds created were easily degradable and converted to carboxyl groups by subsequent oxidation. The results here are consistent with those of Erdogan *et al*., (2007), Lal and Mishra, (2008), Satisha and Devarajan, (2011) and Banik and Sanyal, (2006).

Total acidity

The total acidity of humic acid was ranged from 1.98

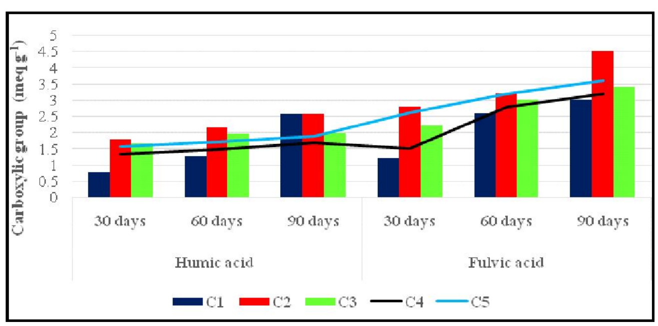

Fig. 2: Changes in the Carboxylic group (COOH) during different stages of enriched insect biomass compost.

Table 2: E_4/E_6 ratio of humic acid and fulvic acid extracted from different insect biomass during different stages of composting.

Insect biomass compost	E_4/E_6 ratio					
	Humic acid			Fulvic acid		
	30 days	60 days	90 days	30 days	60 days	90 days
C1	2.88	3.01	4.17	3.14	3.96	4.57
*C2	4.15 (44.10)	4.43 (47.18)	5.46 (30.94)	6.49 (106.6)	6.83 (72.47)	5.86 (28.23)
*C3	4.09 (42.01)	4.16 (38.21)	4.83 (15.83)	4.32 (37.58)	4.52 (14.14)	5.07 (10.94)
*C4	3.31 (14.93)	3.74 (24.25)	4.34 (4.08)	3.33 (6.05)	5.58 (40.91)	4.72 (3.28)
*C5	3.45 (19.79)	3.88 (28.90)	4.81 (15.35)	4.05 (28.98)	5.65 (42.68)	5.01 (9.63)
SEm ±	**0.022**	**0.22**	**0.021**	**0.055**	**0.045**	**0.020**
CD at 5%	**0.07**	**0.07**	**0.06**	**0.17**	**0.14**	**0.06**

* Insect biomass and FYM mixed in the ratio of 1:4 N equivalent ratios
The figures in parenthesis indicate percent increase and decrease (+/-) in moisture content (%) over farm yard manure (FYM)

meq g^{-1} in (C1) to 3.27 meq g^{-1} in (C2) that of fulvic acid ranged from 3.99 meq g^{-1} in (C1) to 6.66 meq g^{-1} in (C2) at 90th day after composting indicating that fulvic acid has high total acidity than humic acid (Table 1). A similar type of functional group is obtained from humic and fulvic acids by Ramalakshmi, (2011). Fulvic acid has a higher total acidity than humic acid, according to Bannik and Sanyal, (2006). Srilatha (2014), Sanyal (2001), and Sujana Reddy and Rao, (2000) discovered that decreasing molecular weight increased total acidity and enhanced the degree of oxidation of low molecular weight fractions.

Carboxyl groups are more abundant than phenolic hydroxyl groups, implying that carbohydrates and phenolic chemicals in these substances are easily destroyed and change to carboxyl groups upon oxidation (Masaaki *et al*., 1992). The results were similar by Satisha and Devarajan (2011) and Ushashree *et al*., (1989). Humic compounds have a greater acidity due to the presence of ionizable H+ ions with carboxyl and hydroxyl groups, which are typically present in aliphatic rings or aromatic chains of molecules (Schnitzer and Khan, 1972). The current investigation also revealed that carboxylic and phenolic hydroxyl groups were present in varied proportions, which is consistent with the findings of Prasad and Sinha (1981), who claimed that a difference in molecular weight may account for the difference in functional groups.

E_4/E_6 ratio

The E_4/E_6 ratio of humic acid was ranged from 2.88

Table 3: Humic acid index of humic acid and fulvic acid extracted from different insect biomass during different stages of composting.

Insect biomass compost	Humic acid index		
	30 days	60 days	90 days
C1	2.77	1.21	1.33
*C2	1.76(-36.46)	1.80(48.76)	1.93(45.11)
*C3	2.88(3.97)	4.11(239.6)	1.96(47.37)
*C4	1.12(-59.57)	1.57(29.75)	2.02(51.88)
*C5	9.62(247.2)	1.45(19.83)	2.99(124.8)
SEm ±	**0.140**	**0.048**	**0.024**
CD at 5%	**0.43**	**0.15**	**0.08**

* Insect biomass and FYM mixed in the ratio of 1:4 N equivalent ratios
The figures in parenthesis indicate percent increase and decrease (+/-) in moisture content (%) over farm yard manure (FYM)

to 4.15 at 30 days after composting and on the 90th day it ranged from 4.17 to 5.46 recorded in C1 (farmyard manure) and C2 (farmyard manure + silkworm pupae) treatment (Table 2 and Fig. 4). Similarly, that of fulvic acid was ranged from 3.14 to 6.49 at 30 days and on 90th day it was ranged from 4.57 to 5.86 in C1 (farmyard manure) and C2 (farmyard manure + silkworm pupae) treatment and it could be noticed from the data that fulvic acid had higher E_4/E_6 ratio in comparison to the humic acid. It could be because humic acid's carbon atoms have a higher degree of aromaticity. Tahiri *et al*., (2016), Srilatha *et al*., (2013), and Satisha and Devarajan, (2011) all reported that fulvic acid had a slightly higher E4/E6 ratio than humic acid. A low E_4/E_6 ratio of 5.0 indicates a high degree of condensation of aromatic humic components, high molecular weight, and low acidity, all of which are characteristics of the humic acid fraction, whereas a high E_4/E_6 ratio of fulvic acid indicates a low degree of aromatic condensation and the presence of a relatively large proportion of aliphatic structure. These findings are consistent with those of Haddad *et al*., (2015), Petrus *et*

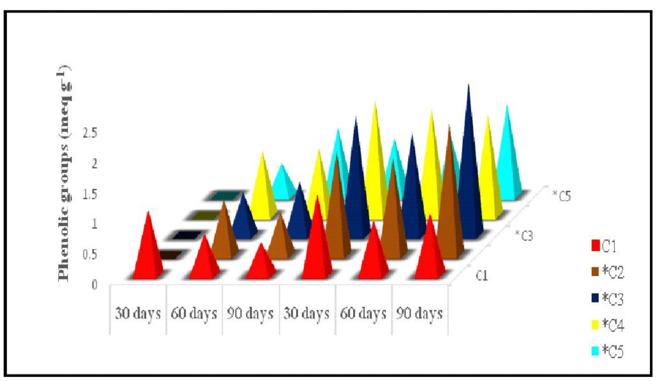

Fig. 3: Changes in Phenolic groups (meq g^{-1}) of humic acid and fulvic acid during different stages of enriched insect biomass compost.

al., (2009), and Banik and Sanyal (2006).

The E_4/E_6 ratio is a reliable and useful measure for determining the aromaticity of humic compounds (Kononova, 1966). E_4/E_6 is a measure of humic acid's aromaticity. E4/E6 was low in treatment (C1- farmyard manure) indicating more aromaticity and the treatment C2 (farmyard manure + silkworm pupae) is also comparable. In the present study, the E_4/E_6 ratio was high in the fulvic acid extracted from silkworm pupae + farmyard manure (5.86) followed by C3 silkworm moth + farm yard manure and fruit fly + farmyard manure treatment (5.07) lower E_4/E_6 ratio was noted in the control treatment (4.57). A greater E_4/E_6 ratio implies a more aliphatic character of the fractions which reflects a low degree of aromatic condensation. Observations comparable to those reported by Pandeya and Singh (2000) and Kadalli *et al*., (2000).

Humic acid index

The humic acid index which is the rate of HA/FA recorded maximum in treatment C5 (9.62) and minimum humic acid index was found in C4 (1.12) at 30 days after composting (Table 3). At maturity, it ranged from 1.33 to 2.99 and was recorded in C1 (farmyard manure) and C5 (farmyard manure + silkworm pupae) treatment. The humification index (HA/FA) can be used to predict maturity and stability. The highest humic acid index values were reported in treatment C5, indicating a degree of humification (high humification). Chefetz *et al*., (1996) state that increasing quantities of humic acid signify the degree of humification and maturity of compost.

Conclusion

Bioconversion was used to convert several insect species such as silkworm pupae, silkworm moth, uzi fly, and fruit fly into compost. The humic acid produced by all enriched compost treatments was greater than the fulvic acid produced. At 90 days, the treatment (C2 - FYM+ silkworm pupae) had higher humic acid and fulvic acid content than the control (Farmyard manure). Humic acid index levels were found to be higher in silkworm pupae enriched compost, followed by moth enriched compost. E_4/E_6 is a measure of aromaticity, and it exhibited lower values in the (C1) condition, which received farmyard manure and cowdung. Higher E_4/E_6 values were observed in treatment C2 silkworm pupae compost. Fulvic acid had a higher total acidity, phenolic group, and carboxylic group when compared to humic acid. The carboxylic group was highest in C2 (FYM + silkworm pupae) of 4.50 meq g^{-1} . The total acidity of fulvic acid was highest in C2 (FYM + silkworm pupae) of 6.66 meq g^{-1} followed by C3 (FYM + silkworm moth). The phenolic group was higher in C3 (FYM + silkworm moth) of 2.50 meq g^{-1}. HS bioactivity can help to reduce fertiliser application rates, increase nutrient efficiency, replace synthetic plant regulators, improve fruit quality, increase water stress tolerance, decrease disease incidence, and promote early growth and flowering, while their chemical composition may be suitable to act as a carrier to introduce beneficial microorganisms into cropping systems. The use of HS as biostimulants in agricultural crops emerges as a major sustainable technology that could combine other agricultural practises to make cropping systems more productive and efficient while also having less negative environmental implications.

Declaration

The authors declare no conflict of interest

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