Efficacy of Agricultural Wastes in the Control of Rice Cyst Nematode (*Heterodera sacchari*)

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Summary

Rice cultivation is endangered by plant parasitic nematodes. Rice cyst nematode (*Heterodera sacchari* Luc & Merni, 1963) is one of the nematode pests which affect the quantity and quality of rice. The use of synthetic nematicide has reduced considerably yield losses incurred by *H. sacchari* infestation; this achievement is associated with environmental damage and occurrence of pesticide residue in food. In an effort to redeem the environment, development of alternatives to conventional nematicide is imperative. Agricultural wastes are renewable source of bio-pesticides if properly processed. The objectives of this research were: to hydrolyze pentoses and convert it to furfural in agricultural wastes; to determine the amount of furfural in 100, 150 and 200 g of agricultural waste; to incorporate the agricultural waste material into the soil as soil amendment; to determine how much furfural was released in the process of acidic/enzymatic hydrolysis of the biomaterial, and to determine the nematicidal effect of furfural in control of rice cyst nematode. Corn cobs (CNCB), rice husks (RCEH) and sorghum husks (SGMH) were digested for furfural production in place of synthetic nematicide carbofuran (CBFN) options in the management of rice cyst nematode. The quantity of furfural in 100, 150 and 200 g of each waste was determined, and the agricultural wastes were applied as soil admixes. The sorghum husk (SGMH) produced the highest furfural amount (0.796). At quantity of 200 g SGMH was significantly ($p=0.05$) better than all other treatments on plant height, number of tillers and rice yield. There was no significant difference of the effect of rate of application (level) on final cyst count in soil and root. Agricultural wastes, especially sorghum husks, can serve as an alternative to the use of synthetic nematicide. Residual furfural was absent in the agricultural waste after harvest. Furfural is quickly broken down by soil microorganisms under aerobic conditions; hence, it is not toxic to the environment.

Key words

pollution, hydrolysate, nematicide, extraction, furfural, *Heterodera sacchari,* sorghum husks

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Introduction

Rice plant (*Oryza sativa* L.) belongs to the Poaceae family. It is one of the leading crops in the world on which about half of the world's population depends. It is the second most cultivated crop after wheat (Normile, 2004). In Nigeria, rice is cultivated in almost all the regions. A total of 77% of the farmed area of rice is rain fed, of which 47% are lowland and 30% upland. The range of rice varieties includes both local ('Ofada', 'Dias', 'Santana', 'Ashawa', 'Yarsawaba' and 'Yarkuwa') and genetically modified traditional African Rice-NERICA (Bayou Farms and Industries Limited, 2009). Rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories for humans worldwide (Smith, 1998). For millions of people, rice is three quarters of their total diet (Thomas, 1997), providing 20% of the per capita energy and 13% of protein needed (Juliano, 1994). It is a good source of protein, but not a complete protein. In addition, by-products of rice (rice hulls, rice bran, broken rice and rice straw) are used in a variety of ways, including horticultural, livestock, industrial, household, building and food products (Rice Growers' Association of Australia, 2004). Pests and diseases, including plant parasitic nematodes (PPN) bring about significant loss in production of rice. Different species belonging to ten genera of PPN are economically important in rice production. Rice grown in different environments are parasitized by different nematode species. Cyst (*Heterodera* spp.), Ufra (*Ditylenchus angustus* Butler, 1913; Filipjev, 1936) and root-knot (*Meloidogyne* spp.) nematodes are major pests of deep water rice (Rao and Jayaprakash, 1977; Varaprasad *et al.*, 2006). Apart from direct crop loss, nematodes also inflict indirect monetary losses resulting from trade restrictions imposed on rice due to the presence of quarantine nematode pests (Varaprasad *et al.*, 2006). Different management strategies have been used to control cyst nematodes on rice with various degrees of success. Application of synthetic nematicides is a simple and effective approach (Sikora and Fernandez, 2005), but with environmental pollution issues. Therefore, an effective and cost saving alternative control option against the rice nematode is highly desired. Thus, investigation of agricultural wastes from crop residues was made for possible control options. Agricultural wastes are abundant, renewable, natural and rich sources of chemicals (Wankasi and Naido, 2012). If these agricultural waste products can be properly recycled into useful products, environmental pollution would be greatly reduced. Because of lignocelluloses in agricultural waste it can be converted into important industrial chemical like furfural that can be used as biopesticide to control nematodes (Grover and Mishra, 1996). The herbicidal properties of aqueous extract from sorghum were reported by Ashraf and Akhlaq (2007). They established a 33% reduction of broad leaf weeds in wheat and this was further affirmed by Ejaz *et al.* (2015), while McKillip and Sherman (1980) indicated that furfural is herbicidal. Corncobs, sorghum husks and rice husks contain cellulose, hemicelluloses and lignin (Barl *et al.*, 1991), and show a great potential as a renewable raw material for producing furfural. Thus, the objectives of this research were (i) to hydrolyze pentoses and convert it to furfural in agricultural wastes and to determine the amount of furfural in 100, 150 and 200 gram of agricultural waste; (ii) to incorporate the agricultural waste material into the soil as soil amendment; (iii) to estimate the residual furfural in the soil after the experiment, and (iv) to determine the nematicidal effect of furfural in the control of rice cyst nematode.

Materials and methods

Collection of agricultural wastes

Agricultural waste products were collected from different locations in Ilorin, Nigeria. The husks of rice was collected from Oja Tun-tun in Ilorin metropolis from a rice milling depot; the sorghum husks was from Sare Local Government Area of Kwara State, and the corn cobs were collected from the Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin. They were spread in the Laboratory to dry for five weeks. The materials were powdered using a STEEL MAN diesel engine with a 7 horsepower capacity, model R175A.

Extraction of furfural

The rice husks, the corn cobs and the sorghum husks in the amount of 100, 150 and 200 g of each was put in a round bottom quick fit distillation flask (1.5 L). A total of 30 ml concentrated tetraoxosulphate (IV) acid (H_2SO_4) as catalyst was added to 400 ml of water (H_2O) and was added to the sample. The mixture was refluxed in a sand bath at 200ºC to convert the pentose in the waste to furfural (Wankasi and Naidoo, 2012). A total of 100 ml of water was added to the mixture, which was then decanted and filtered into a conical flask and the hydrolysate divided into two equal parts of 250 ml each, one for batch extraction (with dichloromethane) and the other half for steam distillation.

Batch extraction

A total of 250 ml of the filtrate was poured in a 500 ml separating funnel and 50 ml of dichloromethane (DCM) was added and shaken thoroughly. The organic phase was separated with a separating funnel, rinsed with little cold water (by returning the organic phase back into the separating funnel), dried (anh. $MgSO_4$), filtered, and concentrated using a rotary evaporator. A clear yellowish liquid furfural was obtained. This process was repeated to extract furfural from the hydrolysate of the each of three agricultural wastes.

Test for furfural

Few drops of the extracted furfural added to acidified (HOAc) distilled aniline gave a blood red colour.

Pot experiment

Unpasteurized loamy soil was collected from the back of the Faculty of Agriculture, University of Ilorin. It was distributed into 48 plastic buckets of 12 liter capacity; five kilograms of soil in each bucket. The population of rice cyst nematode (*H. sacchari*) used was raised under NERICA1 rice variety. The matured rice plants were uprooted and cysts were collected from the roots and soil sample using a modified Fenwick can (MFC) flotation method (Fenwick, 1940). Twenty viable cysts were incorporated into each bucket before planting, to build up nematode populations for the experiment. The estimated number of cysts per pot at planting was twenty five (25). 'NERICA 1' rice variety was planted into the buckets and watered every other day. The experiment had four treatments, (CBFN, SGMH, RCEH and CNCB) at four rates of application (level 0-3), each with three replicates arranged in Randomize Complete Block Design (RCBD). Carbofuran 3G was applied at concentrations of 0.025 g/5 kg soil, 0.042 g/5 kg soil and 0.058 g/5 kg soil. The agricultural wastes were mixed with the soil at 100 g/5 kg, 150 g/5 kg and 200 g/5 kg.

Data collection and statistical analysis

Data were collected from the screenhouse on the following parameters: plant height, number of leaves, and number of tillers; while the final nematode population in 250 g of soil was determined after 14 weeks in the laboratory by the flotation method. The collected data were analysed using a two-way analysis of variance with the generalized linear model. Significant means were separated with new Duncan's multiple range test at 5% level of probability (p=0.05). All statistical analyses were done using IBM SPSS Statistics 21.

Test for residual furfural

The experimental soil was sieved to recover the residual agricultural waste after harvest, in general the materials were completely mixed with soil and are inseparable, but few corncobs were recovered. The recovered corncobs were washed free of soil, air dried and then re hydrolysed. The furfural obtained was then determined.

Results and discussion

Table 1 shows the quantity of furfural obtained from the various agricultural wastes. Sorghum husk (SGMH) had the highest overall yield. The main effect of treatments and level of application of treatments on plant height, number of tillers, and number of leaves are depicted in Figure 1-6. Rice plants grown in pots amended with SGMH were significantly taller (p=0.05) as opposed to the other treatments. At the fifth week after planting, plants in SGMH amended pots were 32.23 cm high, while plants treated with corncob

Table 1. Furfural Yield from agricultural wastes

Agr. Waste*/Quantity	100 g	150 g	200 g
CNCB	0.332 g	0.498 g	0.664 g
RCEH	0.044 g	0.066 g	0.088 g
SGMH	0.398 g	0.597 g	0.796 g

* CNCB - Corn cobs, RCEH - Rice husk, SGMH - Sorghum husk

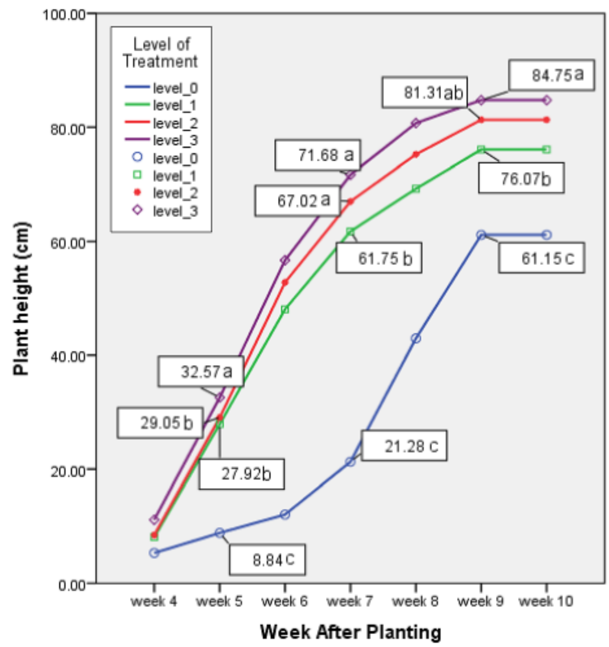

Figure 2. Effect of CBFN, SGMH, RCEH and CNCB treatment levels (dosage 100 g, 150 g and 200 g) on height of rice plants

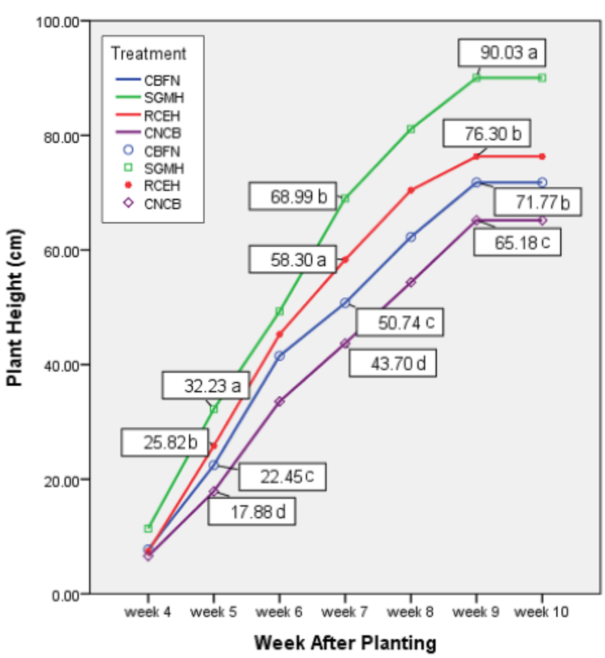

Figure1. Effect of treatments on height of rice plants

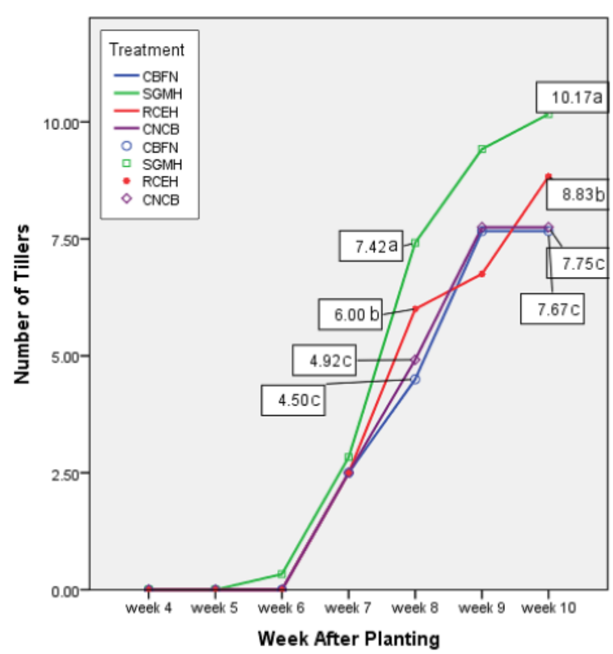

Figure 3. Effect of treatments on tillering of rice plants

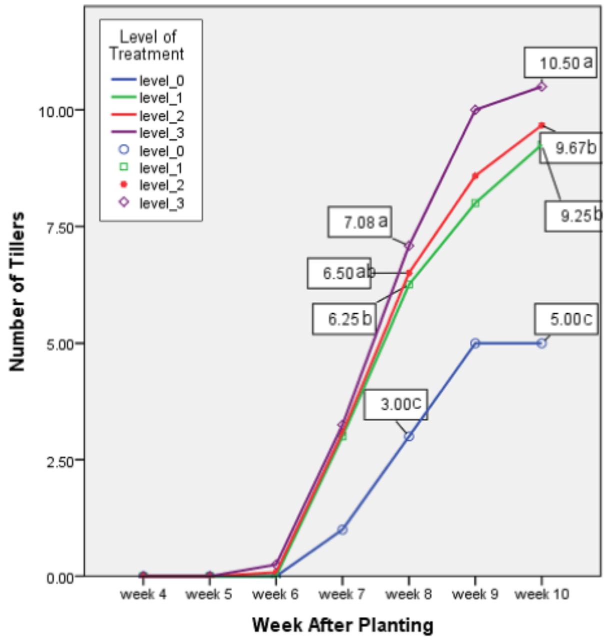

Figure 4. Effect of CBFN, SGMH, RCEH and CNCB treatment levels (rate 100 g, 150 g and 200 g) on tillering of rice plants

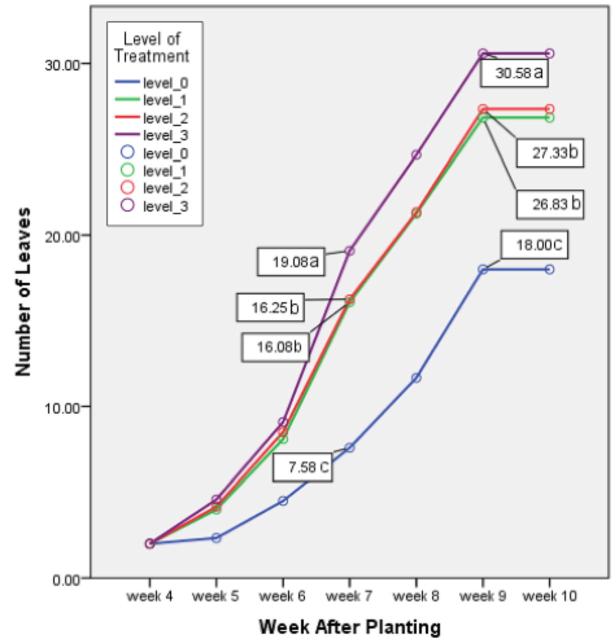

Figure 6. Effect of CBFN, SGMH, RCEH and CNCB treatment levels (rate100 g, 150 g and 200 g) on number of leaves of rice plants

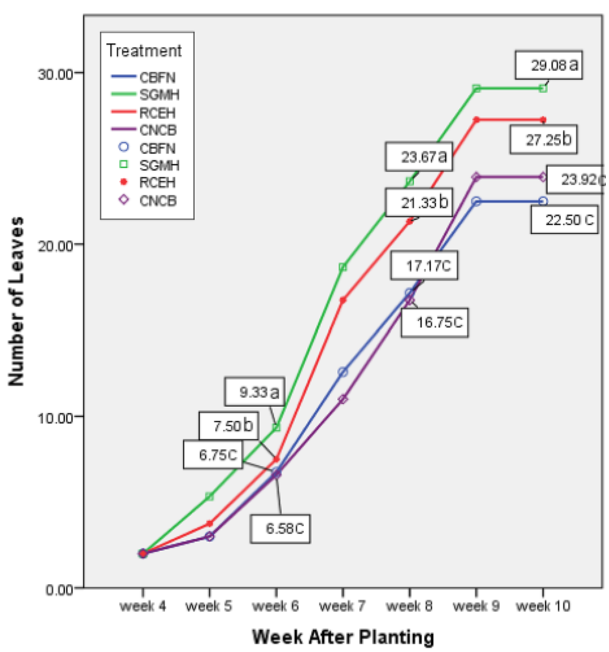

Figure 5. Effect of treatments on number of leaves of rice plants

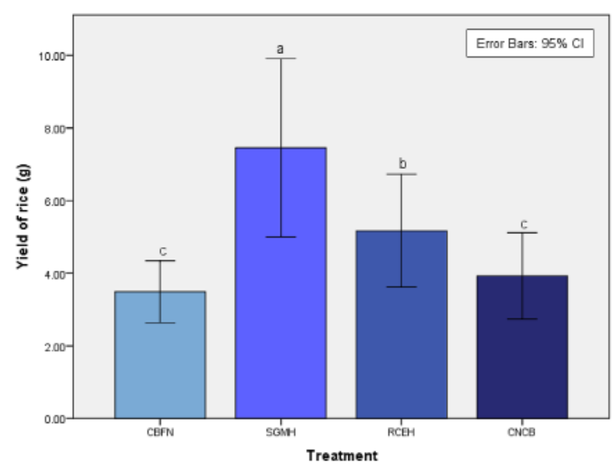

Figure 7. Effect of treatments on yield of rice plants

husk (CNCB) were 17.88 cm high. At the 10th week after planting, SGMH amended plants had the highest plant height of 90.03 cm, the highest number of tillers (10.17) and the highest number of leaves (29.08 cm) in comparison with plants treated with corncob husk (CNCB) and rice husk (RCEH). A significant (p=0.05) reduction was also observed in the cyst count in pots amended with SGMH; cyst count was lower in relation to other treatments (Fig. 9). The level of application was equally significant on the performance of the rice plants. The highest level (200 g/ level 3) produced significantly taller plants, had the highest number of tillers and number of leaves. Rice yield was also significantly (p=0.05) higher in plants which received the highest level of soil amendment (Fig. 8). However, there was no significant difference among the different levels of treatment application on cyst count (Fig. 10). The activity of carbofuran (CBFN) on number of tillers and yield of rice was not significantly different from corncob husk (Fig. 3 and 7). In most cases SGMH was significantly more effective than CBFN.

In this research it was observed that furfural from agricultural waste was effective in the control of the rice cyst nematode (*H. sacchari*). Furfural, a naturally occurring aromatic aldehyde, has been

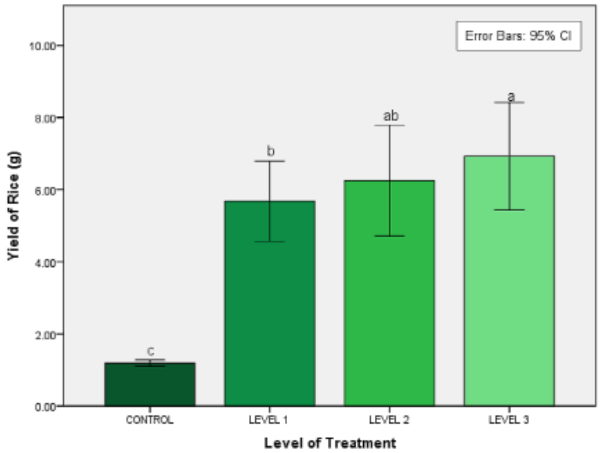

Figure 8. Effect of CBFN, SGMH, RCEH and CNCB treatment levels (rate 100 g, 150 g and 200 g) on yield of rice plants

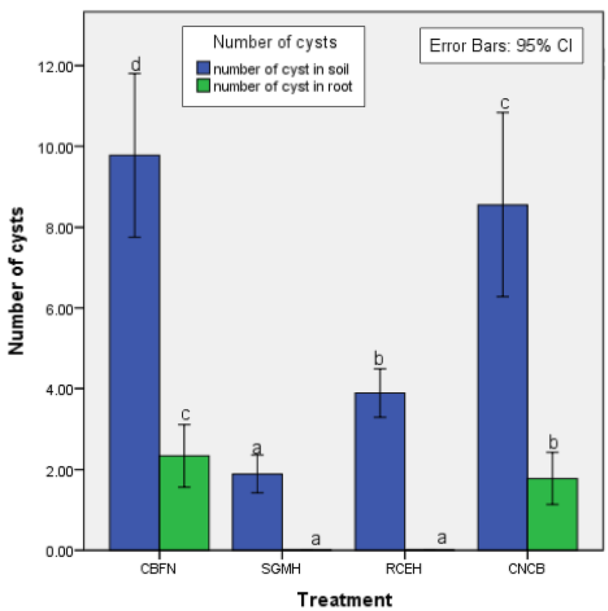

Figure 9. Effect of treatments on cyst count in root and soil

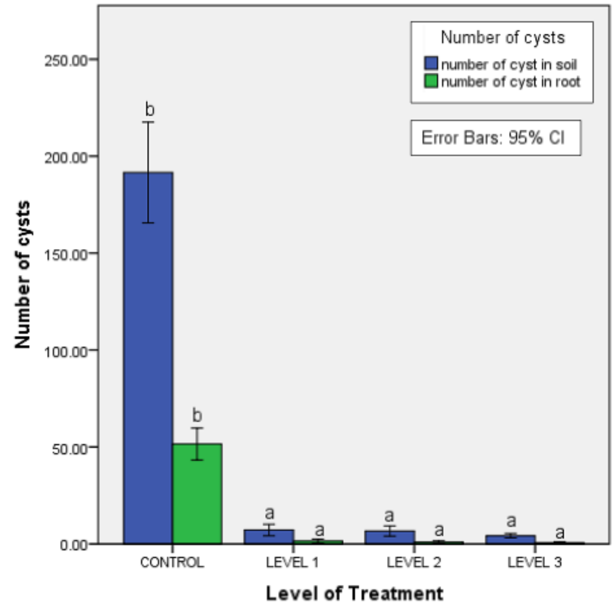

Figure 10. Effect of CBFN, SGMH, RCEH and CNCB treatment levels (rate 100 g, 150 g and 200 g) on cyst count in root and soil

reported to be nematicidal against nematodes like *Meloidogyne arenaria* (Neal, 1889; Chitwood, 1949), *Pratylenchus brachyrus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, *Heterodera glycines* Ichinohe, 1952 and *Helicotylenchus dihystera* (Cobb 1892) Sher 1961 (Rodríguez-Kábana *et al.,* 1993; Bauske *et al*., 1994). Furfural is also indicated to be an effective nematicide against *Belonolaimus longicaudatus* (Rau, 1958) in turf grass (Luc and Crow, 2013). Aromatic aldehydes such as benzaldehydes and cinnamaldehyde have been reported as having insecticidal properties on stored grains (Ishibashi and Kubo, 1987). Observations in this study show that sorghum husk, which produced the highest quantity of furfural during the extraction process, significantly increased rice yield, number of tillers and plant height; an observation which could be associated with the quantity of furfural present in the sorghum husk (SGMH). After harvest, no significant amount of furfural was left in the corncob (CNCB). This is largely due to the amount of furfural consumed as bio nematicide. Other agricultural wastes materials like the RCEH and SGMH were totally mixed with the soil and inseparable, thus explaining that the materials have biodegraded.

Conclusion

In this investigation significant nematicidal action was displayed by sorghum husk (SGMH). We can infer that the quantity of furfural produced under laboratory experimental conditions during hydrolysis is most probably comparable to the quantity released by the agricultural wastes after incorporation into the soil. SGMH has the highest quantity of furfural, which explains the observed bioactivity of SGMH. The use of agricultural wastes with lignocellulosic properties as soil admixes will go a long way in the management *H. sacchari*. There was no residual furfural after harvest, indicating the quick bio degradable characteristics of the agricultural wastes. To find out accurate efficiency of SGMH under field conditions further research should be conducted.

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