Chem. Vol. 67, SI: M. R. Mahran, pp.511– 518 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Utilizing Biowaste-Derived Chemicals in Designing, Preparing, and Evaluating the Biopesticidal Activity of A New Formulation For Controlling Root Rot and Root Knot Infection in Green Beans

Galal A.M. Nawwar [1], Hoda Sabry Othman *[1], Mokhtar Abdel-Kader [2], Nehal S. El-Mougy [2]

[1] Green Chemistry Department, National Research Centre, Cairo, Egypt.
[2] Plant Pathology Department, National Research Centre, Cairo, Egypt.

In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

The current study investigates the use of alkaline black liquor (ABL), a byproduct of the rice straw solar pulping process, to create a bioactive formulation that can inhibit the growth of soilborne plant pathogens. ABL was first treated to precipitate lignin, silica, and fatty acids as a calcium complex (Ca-LSF). The resulting highly alkaline effluent after chemical analyses was used to hydrolyze chicken feather waste to its amino acid constituents. The resulting new protein hydrolysate was chemically analyzed and then added to the Ca-LSF complex, affording a novel formulation with slow-release nitrogen, carbon, and bioactive phenolic sources to be evaluated against soilborne pathogenic fungi Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, and Sclerotium rolfsii, as well as the nematode Meloidogyne incognita, the causal organisms of wilt, root rot, and root knot diseases. The prepared formulation showed a strong inhibitory effect against R. solani and affected the viability of M. incognita. This effect increased with increasing concentration, up to 4%, which completely inhibited fungal growth. The presented safe, cost-effective, and eco-friendly formulation could be used to control a wide range of soilborne plant pathogens; moreover, it presents a complete recycling of two hazardous biowastes via zero-waste processes.

Key words:Biowaste-derived chemicals, Rice straw, Biocide, root rot, root knot.

1. Introduction

Egypt produces a large amount of rice straw annually. Only about 20% of it is used in industries such as paper, fertilizers, ethanol production, and livestock feed. The remaining 80% is mostly discarded by burning it in the open, which creates black clouds and pollutes the air. Various efforts have been exerted to reduce environmental pollution, for example, the chemical refining of agro-wastes to produce bioactive chemicals. In this context, the pulping of rice straw [1] results in the production of black liquor containing lignin, silica and fine chemicals like ferulic and coumaric acids as well as other phenolics (Fig.1). The refining of such chemical feedstock using ecofriendly methods has significance and relevance to the field of green chemistry. Also, pulping produces cellulosic pulp, which could be utilized in the production of important industrial chemicals such as microcrystalline cellulose and carboxymethyl cellulose [2, 3].

It is worth mentioning that chemical pulping of rice straw under conventional drastic conditions (high temperature and pressure) is associated with different side reactions of these components, which lead to several condensed products and a dark brown color, affording toxic liquor. On the other hand, mild conditions, such as in the alkaline solar pulping process, lead to nontoxic black liquor that can be easily refined [4-6]. This black liquor has been chemically modified and fermented recently using a variety of green methods to produce useful enzymes such as xylanase [7], *Aspergillus awamori EM66 levansucrase* [8], and β-glucosidase [9]. In addition, it has been used to precipitate new metal (lignin/silica/fatty acids) complexes that have been successfully used as antioxidants, fillers, and reinforcing agents in green rubber composites [6, 10-12].

*Corresponding author e-mail: hoda_sabry2@yahoo.com
Receive Date: 25 December 2023, Revise Date: 29 January 2024, Accept Date: 05 February 2024
DOI: 10.21608/ejchem.2024.257757.9068

Plant diseases are a recurring problem in many areas. They can spread rapidly and are difficult to cure once they have taken hold. This is why most management methods focus on preventing diseases from occurring in the first place.

Beans (Phaseolus vulgaris L.) are one of the most important food legumes in the world, grown for both local consumption and export [13]. They are susceptible to a number of diseases, including wilt, root rot, and leaf spot. The most common soilborne fungi that cause these diseases are *Fusarium solani*, *Sclerotium rolfsii*, and *Rhizoctonia solani* [14, 15]. Nematodes can also damage beans by feeding on their roots and reducing nutrient uptake, which can lead to root knot disease. These diseases can have a significant negative impact on plant growth and crop yield. Root rots can affect seedlings throughout the growing season, from emergence to the seedling stage. They can also infect seeds before emergence, which can lead to pre-emergence infection and the need to replant missed hills or dead plants. Root-knot nematodes (*Meloidogyne spp.*) are known to cause significant damage to crops all over the world. They are found in a wide range of climatic conditions and can attack a variety of crops. The damage caused by these nematodes is often severe and can lead to significant crop losses [16-18]. The disease caused by *Meloidogyne spp.* is often the only, or one of the few, nematode diseases that farmers are aware of, as the nematodes' subterranean activities are not easily visible. However, the symptoms of the disease, such as stunted growth, wilting, and yellowing of leaves, are often unmistakable [19, 20].

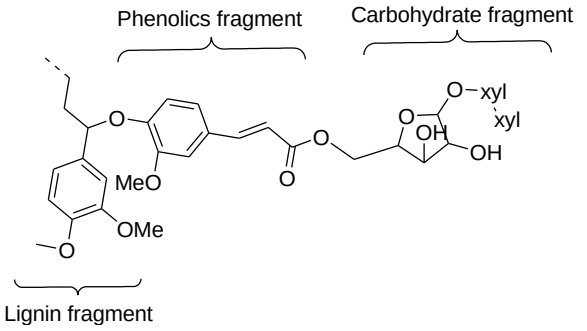

Fig. (1) Lignin/Phenolics/Carbohydrate complex in rice straw.

There are a variety of methods to control *Meloidogyne spp.*, including crop rotation, resistant varieties, and nematicides. However, no single method is completely effective, and a combination of methods is often necessary to manage these nematodes. Researchers have been working to find effective substances that can control root knot disease. Overuse of synthetic pesticides results in crop contamination and eutrophication when they slowly seep into water systems through rainfall. Environmentalists are increasingly supporting a number of green bio-approaches that boost crop productivity and protect crops.

Biomass-derived substances are emerging as a promising option without the dangers of using synthetic pesticides, as they have been shown to be effective against nematodes and relatively safe for the environment [21]. The effectiveness of ammonia against soilborne microorganisms has been widely studied. Many ammonia-releasing compounds, such as ammonium hydroxide, ammonium phosphate, and ammonium bicarbonate, have been tested, and they all showed promising nematicidal activity in pots, with ammonium hydroxide being the most effective [22]. In addition to its toxicity to fungi, ammonia also **increases soil pH and may stimulate microbial activity that can help suppress pathogens [23-26].**

Since **ammonia is supposed to be the active agent released from urea,** Rodríguez-Kábana and King [27] found that urea can be used to control root knot nematodes when applied to the soil at a rate of 0.4 g/kg. **However**, this rate can be phytotoxic to plants. When urea is mixed with carbon-rich blackstrap molasses, the phytotoxicity is eliminated, nematode populations are reduced, and plant weight and height are increased. This suggests that adding nitrogen in the form of urea is an effective way to control root knot nematodes. Huebner et al. [28] found that soil amended with carbon-rich lignocellulosic waste had more nematicidal efficacy when urea was also added to the soil.

In chicken processing factories, feathers are generated in significant quantities as a byproduct. Too frequently, they are burned or disposed of in landfills, which causes waste disposal issues and environmental contamination hazards. An alternate method for producing a marketable final product that clearly benefits the primary producers in terms of economic and environmental management **methods** is the biodegradation of chicken processing waste [29]. Feather meal contains 15% nitrogen and is inexpensive and readily available. It has the potential to be used as a biofertilizer [30]. The breakdown product of chicken feathers may produce a suitable quantity of tryptophan, which is essential for the production of indole-3-acetamide, a plant growth regulator that mediates the synthesis of indole acetic acid (IAA) [31].

As our program aims to develop an environmentally friendly approach to synthesizing bioactive products [32-36], the black liquor obtained from the solar pulping of rice straw [6] was used in the current study to chemically digest chicken feather waste to design, prepare, and **evaluate** the biopesticidal activity of a newly prepared formulation.

2. Materials and methods

2.1. Preparation of the solar pulping black liquor

The black liquor from the solar-pulping of rice straw (pH=10) was prepared as **previously** described [6].

2.2. Preparation of the treated black liquor:

The obtained black liquor was treated by adding 5 grams of calcium oxide to 1 liter of black liquor (pH = 10) while stirring for 1 hour. The mixture was then left overnight and filtered to obtain 10 grams of calcium (lignin/silica/fatty acids) complex (Ca-LSF) [6]. The effluent (pH = 12) was analyzed for its chemical constituents (Table 1), and its total phenolic content was determined (Table 2).

2.3. Preparation of chicken feather hydrolysate:

The effluent (pH = 12) was then used in the alkaline hydrolysis of 50 grams of chicken feathers for 3 hours at 90 ºC, followed by acidification with 5% sulfuric acid till pH = 4, filtration (to get rid of sodium sulfate), and analysis for its chemical constituents (Table 1).

2.4. Preparation of the tested formulation:

The prepared hydrolysate (75 grams) was mixed with 10 grams of Ca-LSF complex, yielding the tested formulation (85 grams). The chemical composition of this formulation is shown in Table 1.

2.5. Tested material and microorganisms:

The pathogenic fungi *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, and *Sclerotium rolfsii*, which cause wilt and root rot diseases, as well as the culture of the pathogenic nematode *Meloidogyne incognita*, which causes root knot disease, were kindly provided by the Plant Pathology Department of the National Research Centre in Egypt.

2.6. EDAX analysis:

EDAX was used to characterize the elemental composition of the hydrolysate. It was performed using Quanta FEG 250 scanning electron *microscope* (FEI Co., Hillsboro, OR, USA) with an attached EDAX unit.

2.7. Total phenolic content:

The total phenolic content (TPC) of the effluent was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [37] with some laboratory modification. A sample of the effluent residue after its evaporation under vacuum was used in the analysis, dissolved in methanol. The reaction mixture was prepared by mixing 0.5 ml of effluent solution, 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water, and 2.5 ml of 7.5% $NaHCO_3$. A blank was prepared, containing 0.5 ml of methanol, 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water, and 2.5 ml of 7.5% $NaHCO_3$. After 90 minutes of incubation at 30°C in a thermostat, the absorbance was measured with a spectrophotometer at λ max = 765 nm. The sample was prepared in triplicate for each analysis, and the mean value of absorbance was obtained. The calibration line was created by repeating the process with the standard gallic acid solution. The concentration of phenolics was determined (in mg/mL) from the calibration line using the measured absorbance, and then the composition of phenolics was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

2.8. Effect on *Fungal Linear Growth:*

The prepared formulation was evaluated against the growth of the above-mentioned soilborne pathogenic fungi at different concentrations (1%, 2%, 3%, and 4%). A certain weight of the formulation was added to autoclaved melted potato dextrose agar (PDA) medium and carefully mixed by gentle swirling to ensure that the concentration was evenly distributed before pouring into 9 cm diameter Petri dishes. After the agar solidified, a 5 mm disk was cut from the edge of an actively growing colony of each tested fungus and placed individually in the center of each Petri dish. A separate PDA plate without the formulation powder was used as the control treatment. All Petri dishes were incubated at 25±1°C, and the radial growth of the fungi was observed daily until the colonies completely covered the medium in the control set. The diameter of the mycelial growth was then measured in millimeters. Three replications were used for all treatments, and the experiments were repeated three times. The reduction percentage in fungal growth was calculated relative to its growth in the control treatment using the following formula:

$$\textit{Percentage of mycelial inhibition} = \frac{dc - dt}{dc} * 100\%$$

Where dc is the mean colony diameter in control sets and dt is the mean colony diameter in treatment sets.

2.9. Effect on total fungal count in agricultural soil

Randomized selected different cultivated soil samples were collected from a heavily infested natural field with soilborne pathogens located at the Research and Production Station, National Research Centre, Beheira Governorate, Egypt. The samples were mixed together and then divided into two subsamples of 1 kilogram each. One subsample was amended with 5% formulation powder, while the other subsample was left untreated. Both subsamples were water-irrigated to field holding capacity and left overnight at room temperature. The total fungal counts in the two prepared soil samples were then

determined using the plate count technique, as described by Alexander [38]. This procedure was repeated three times to ensure the reliability of the results.

2.10. Effect on the viability of Meloidogyne incognita J2 phase:

The efficacy of the tested formulation on the total number of J_2 (second stage juveniles) of the nematode *Meloidogyne incognita* was evaluated using the plate count technique with the aid of a microscope, as described by Ashoub and Amara [39]. The tested formulation was investigated at three concentrations (1%, 3%, and 5%) using distilled water. A certain number (100 larvae) of live moving larvae were placed into Petri dishes (9 cm in diameter). The prepared solutions of the tested formulation were then poured individually into these plates. A blank solution, without any formulation powder, was used as the control treatment. Five replicates were used for each treatment. All plates were incubated at room temperature (22–27°C) and examined microscopically after 24, 48, and 72 hours. The percentage of mortality or unmoved larvae was calculated using the following formula:

Mortality (%) = number of dead larvae in treatment * 100% Total larvae number

At the end of the experiment, after 72 hours, the larvae were transferred to distilled water and left for 24 hours. They were then examined, and the final number of dead larvae in each treatment was calculated. The recovered larvae, which had detached themselves from the formulation powder, were not included in the final count.

3. Results and Discussion

The tested formulation was selected to be prepared based on the reported studies that revealed that ammonia gas fumigation can effectively suppress *Fusarium* wilt [24], and that ammonium bicarbonate has been shown to be effective against soil-borne diseases such as southern stem rot, Sclerotinia blight of peanut [25], and *Fusarium mycelia* [26].

The toxicity of ammonia to susceptible organisms may be due to the passive diffusion of the non-ionized NH_3 species through cell membranes and the attraction-repulsion effects of ammonia on entomopathogenic nematodes. These nematodes are parasites of insects and can be used to control insect pests. Ammonia can attract nematodes to a new host, but it can also repel them [40, 41]. The exact mechanism of this attraction-repulsion is not fully understood. Based on the aforementioned literature, a design for the presented formulation was established to produce an effective pesticidal formulation.

3.1. Design of the Chemical processes:

A mild alkaline solar pulping process produces nontoxic black liquor (pH = 10) [6]. Treating this black liquor with calcium hydroxide liberated the sodium hydroxide initially used in pulping *via* cation-exchange reaction, which in turn increased the hydrolysis efficiency of the treated effluent (pH = 12) to digest more chicken feather at 90 °C. Also, the total phenolic content of the treated effluent (Table 2) made the digesting process less vulnerable to oxidation.

The addition of the obtained calcium complex to the hydroysate (pH = 4) neutralized the sulfate anions and formed calcium sulfate as a mending agent for the soil. Thus, the designed formulation (Table 1) contained a significant amount of nitrogenous compounds (about 50%), which were derived from the digested feather ketenes. These nitrogenous compounds were released and degraded slowly, affording ammonia, rendering them beneficial for plant growth and enhancing biopesticide activity. The formulation also contained about 39% of hemicellulose and cellulose, which helps to reduce its phytotoxicity. Additionally, the hemicelluloses/cellulose can be biodegraded under field conditions, releasing active pesticidal chemicals such as furfural [42], along with the bioactive phenolics of rice straw, which are known for their pesticidal activity [43].

As the formulation is further enriched with the Ca-LSF complex, which contains known polyphenolic bioactive lignin as an antioxidant and pesticide [6, 10, 44], this formulation has the potential to be used as a dual-function biopesticide and biofertilizer.

Table (1) Chemical composition of the treated black liquor, chicken feathers hydrolysate, and the tested formulation:

Component	Treated black liquor effluent	Black liquor-chicken feather hydrolysate	The tested formulation
% on air dried basis (moisture)	9.43	1.37	5.88
hydrolysable protein (ammonia &Nitrogen source)	15.10	49.57	28.80
Crude fibers (hydrolysable lignin)	1.50	1.12	6.84
Crude fat	0.87	3.53	1.51
Ash(silica &inorganics salts)	26.89	6.11	32.22
Total carbohydrate (hemicellulose/cellulose as carbon sources)	46.21	38.30	24.75

Table (2) the total phenolic content of the black liquor effluent:

Extract	Conc (mg eq GA/ g)		SD	SE
Treated effluent	178.1	±	2.5	1.4

The result was given as the mean ±SD for at least three replicates of the sample; (SD) and (SE) indicate the standard deviation and the standard error, respectively.

3.2. EDAX of formulation:

It could be clearly shown from Table 3 that the elements comprising the tested formulation, i.e., carbon and oxygen, were in the expected ratio, with a considerable amount of associated silica (14.29%), as it was based on rice straw. Nitrogen was also detected (3.09%), which confirmed the presence of the nitrogenous components. Moreover, the presence of the expected cations, such as Ca^{++} and K^{+}, could also be verified. However, the high content of sodium cations in the alkaline black liquor was diminished in the examined formulation as intended due to the known hazardous effect of sodium salinity on the plant.

Table (3) EDAX analysis of the tested formulation:

Element	Weight %	Atomic %	Net Int.	Error %
C K	20.64	30.51	2.22	30.04
N K	3.09	3.95	1.33	82.42
O K	46.57	51.68	8.51	19.34
NaK	1.1	0.85	0.51	79.3
SiK	14.29	9.04	18.83	11.04
S K	1.37	0.76	1.53	68.02
K K	4.94	2.24	4.74	29.03
CaK	11.09	4.91	8.65	19.78

3.3. Effect on fungal linear growth:

The effectiveness of the prepared formulation against the growth of the tested soilborne pathogenic fungi was evaluated under in vitro conditions (Fig. 2). Table 4 shows that the prepared formulation began to affect the growth of the tested fungi at a concentration of 3%, with the highest growth reduction being 72.4% to 82.6% at a concentration of 4%.

3.4. Effect on total fungal count in agricultural soil

The inhibitory effect of the tested formulation was assayed in a cultivated soil sample treated with a 5% formulation, and then the total fungal counts per gram of dry soil were calculated. Table 5 shows that the total fungal counts in the treated soil (12 $x10^6$ cfu/g) were drastically reduced by 64.7% compared to the untreated soil ($34x10^6$ cfu/g). This reduction could be attributed to the release of ammonia throughout the soil particles when the formulation was incorporated into the soil. The fungicidal and nematicidal effects of ammonia have been reported previously. Ammonia was found to inhibit the growth and spore germination of soilborne fungi [45]. It also exhibited a nematicidal effect against root knot nematodes.

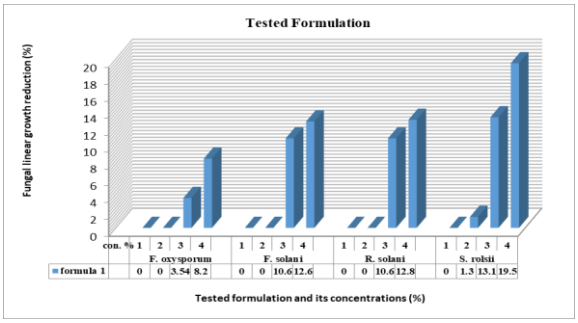

Fig. (2) Reduction (%) in linear growth of tested fungi in response to different concentrations in vitro.

3.5. Effect on the viability of Meloidogyne incognita J2

The effect of the tested formulation on the viability of the *nematode Meloidogyne incognita* J_2 was evaluated under in vitro conditions (Fig. 3). Table 6 shows that the number of dead nematode larvae increased with increasing the concentration of the formulation and with prolonged exposure time. The highest percentage of dead nematode larvae was observed at 5% formulation, which recorded 73%, 98%, and 100% mortality after 24 hours, 48 hours, and 72 hours, respectively. In contrast, no mortality was observed in the control group (sterilized distilled water).

Table (4): Linear growth of some fungi in response to different concentrations of the tested formulation in vitro

Tested formulate	Growth (mm) of tested fungi															
	F. oxysporum				*F. solani*				*R. solani*				*S. rolsii*			
	Concentration (%)															
	1.0	2.0	3.0	4.0	1.0	2.0	3.0	4.0	1.0	2.0	3.0	4.0	1.0	2.0	3.0	4.0
1	90.0 ±0.1g	90.0 ±0.1g	86.8 ±0.1g	82.6 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	80.4 ±1.9f	78.6 ±1.9e	90.0 ±0.1g	90.0 ±0.1g	80.4 ±1.9f	78.4 ±1.9e	90.0 ±0.1g	88.8 ±1.9f	78.2 ±1.9e	72.4 ±1.9e
Control	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g	90.0 ±0.1g

Means ± standard deviations within a column followed by the same letter are not significantly different at $P < 0.05$

Table (5): Effect of the tested formulation at different concentrations on the growth of some pathogenic soilborne fungi

Soil sample	Total fungal count (cfu/g) dry soil	Reduction (%) in fungal counts
Treated soil sample with formulation powder	$12x10^6$	64.7
Untreated soil sample	$34x10^6$	--

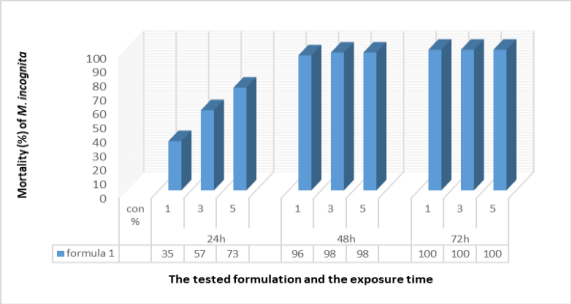

Fig. (3) Reduction in J_2s mortality of *Meloidogyne incognita* in response to different concentrations of the tested formulation

The pesticidal activity of the tested formulation can be attributed mainly to the presence of polyphenolic compounds from hydrolyzable lignin residues under pulping conditions [46, 47]. These compounds synergize with the hydrolyzable feather ketene protein amino acids, which slowly degrade and release the known bioactive ammonia [48, 49]. Moreover, the presence of the known rice straw natural phenolic components, including cinnamic, ferulic, coumaric, and hydroxy benzoic acids derivatives that are liberated from the hydrolysis of their glycosidic linkages [50], along with the hemicellulose content as a carbon source, all contribute to the pesticidal activity of the presented formulation [51].

4. Conclusion

The importance of the current study is to explore the potential pesticidal activity of the chemical derived from rice straw black liquor and its chicken feather hydrolysate *via* green methods to be used as a protective formulation that can inhibit harmful soilborne plant pathogens and prevent them from causing damage. Based on the above-mentioned results, the action of the designed chemical processes allows for liberation of active biochemicals in the tested formulation. Moreover, further decomposition of these constituents in soil is expected to produce a wide range of known nematicidal agents that also have significant fungicidal activity in the soil. This led to a reduction in the causal pathogens of root rot and the root knot nematode Meloidogyne incognita, as well as its galling and egg masses.

Also, this study presents useful rapid removal of the recalcitrant feather and rice straw wastes via utilizing them to produce acceptable land-use products.

5. Conflict of interest

The authors have declared that no conflict of interest.

6. Acknowledgement

The authors are grateful to the Administration Board of the Researches and Production Station, National Research Centre, Beheira governorate for providing the facilities that made this work possible.

7. References

1- Rodríguez, A., Moral, A., Serrano, L., Labidi, J., & Jiménez, L. Rice straw pulp obtained by using various methods. *Bioresour. Technol*. 99, 2881-2886 (2008).

2- Huang, G., Liang, X., Chen, Z., & Li, C. Environmentally friendly pulping process for rice straw to eliminate black liquor discharge. *Tappi J.* 10, 39-44 (2011).

3- Pham, C. D., Ly, T.B., Dang, M.D.T., Tran, K.D., Vo, N.T., Le, T.M., Do, N.H.N., & Le, P.K. The utilization of black liquor from rice straw pretreatment stage on the syntheses of carbon-based materials using in anodes ion-lithium batteries production. *J. Mater. Sci.: Mater. Electron*. 34, 335 (2023).

Table (6): Effect of the tested formulation at different concentrations on the viability of J_2 nematode *Meloidogyne incognita*

	Concentrations (%)	Exposure time					
		24 hrs		48 hrs		72 hrs	
		Live	Dead	Live	Dead	Live	Dead
The tested formulation	1.0	65	35	2	96	0	100
	3.0	43	57	2	98	0	100
	5.0	27	73	4	98	0	100
Control		100	0	100	0	100	0

4- Ibrahim, M. M., El-Zawawy, W. K., & Nawwar, G. A. Modified kaolin and polyacrylic acid-g-cellulosic fiber and microfiber as additives for paper properties improvements. *Carbohydr. Polym*. 88, 1009-1014 (2012).

5- El-Maghraby, S., Nawwar, G. A., Bakr, R. F., Helmy, N., & Kamel, O. M. Toxicological studies for some agricultural waste extracts on mosquito larvae and experimental animals. *Asian Pac. J. Trop. Biomed.* 2, 558-563 (2012).

6- Abdel Zaher, K. S., Swellem, R. H., Nawwar, G. A. M., Abdelrazek, F. M., & El-Sabbagh, S. H. Proper use of rice straw black liquor: lignin/silica derivatives as efficient green antioxidants for SBR rubber. *Pigment. Resin Technol*. 43, 159-174 (2014).

7- Shata, H. M. A. H., El-Deen, A. M. N., Nawwar, G. A. M., & Farid, M. A. F. Xylanase production by mixed culture using crude hemicellulose from rice straw black liquor and peat moss as an inert support. *J. Appl. Biol. Chem*. 57, 313-320 (2014).

8- Mostafa, F. A., Abdel Wahab, W. A., Salah, H. A., Nawwar, G. A., & Esawy, M. A. Kinetic and thermodynamic characteristic of Aspergillus awamori EM66 levansucrase. *Int. J. Biol. Macromol*. 119, 232-239 (2018).

9 - Shata, H. M., El-Deen, A. M. N., Nawwar, G. A., & Farid, M. A. β-Glucosidase production by mixed culture using crude hemicellulose from rice straw black liquor and peat moss as an inert support. *Egypt. Pharm. J.* 13, 121-129 (2014).

10- Abdel Zaher, K. S., El-Sabbagh, S. H., Abdelrazek, F. M., & Nawwar, G. A. Utility of zinc (lignin/silica/fatty acids) complex driven from rice straw as antioxidant and activator in rubber composites. *Polym Eng Sci*. 59, E196-E205 (2019).

11- Othman, H. S., El-Hashash, M. A., El-Sabbagh, S. H., Ward, A. A., & Nawwar, G. A. A copper-(lignin/silica/fatty acids) complex as an antioxidant/electrical conductivity agent for rubber composites (Part iii). *Pigment. Resin Technol*. 51, 42-52 (2022).

12- Othman, H. S., El-Sabbagh, S. H., & Nawwar, G. A. Utility of copper (lignin/silica/fatty acids) complex derived from rice straw as antioxidant/hardening and fluid resistant agent in nitrile-butadiene rubber composites (PART IV). *Pigment. Resin Technol*.51, 518-527 (2022).

13- Baudoin, J. P., Camarena, F., Lobo, M., & Mergeai, G. Breeding Phaseolus for Intercrop Combinations in Andean Highlands. *Broadening the genetic base of crop production*, 373-384 (2001).

14. Abdel-Kader, M. M. Field application of *Trichoderma harzianum* as biocide for control bean root rot disease. *Egypt. J. Phytopathol*, 25, 19-25 (1997).

15- El-Mougy, N. S., El-Gamal, N. G., & Abdel-Kader, M. M. Control of wilt and root rot incidence in *phaseolus vulgaris* l. by some plant volatile compounds. *J. Plant Protec. Res.* 47, 255-265 (2007).

16- Sasser, J. N., & Freckman, D. W. A world perspective on nematology: the role of the society, 7-14. *Vistas on Nematology (Eds. JA Veech & DW Dickson)*. Society of Nematologists, Hyattsville, Maryland, USA (1987).

17- Jones, J. T., Haegeman, A., Danchin, E. G. J., Gaur, H. S., Helder, J., Jones, M. G. K., Kikuchi, T., Manzanilla-Lopez, R. H., Palomares-Rius, J. E., Wesemael, W. M. L., & Perry, R. N. Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathol.* 14, 946-961 (2013).

18- Abd-Elgawad, M. Yield losses by phytonematodes: challenges and opportunities with special reference to Egypt. *EJAJ*.13, 75-94 (2014).

19- Hussain, M. A., Mukhtar, T., & Kayani, M. Z. Assessment of the damage caused by *Meloidogyne incognita* on okra (Abelmoschus esculentus). *J. Anim. Plant Sci.* 21, 857-861 (2011).

20- Abd-Elgawad, M. M. M. Phytonematode damage, economic threshold and management with special reference to Egypt. *EJAJ*. 12, 159-176 (2013).

21- Souto,, A.L., Sylvestre, M., Tölke, E.D., Tavares , J.F., Barbosa-Filho , J.M., & Cebrián-Torrejón, G. Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. *Mol*., 26, 4835 (2021).

22- Oka, Y., & Pivonia, S. Use of ammonia-releasing compounds for control of the root-knot nematode *Meloidogyne javanica. Nematol*. 4, 65-71(2002).

23- Setua, G. C., & Samaddar, K. R. Evaluation of role of volatile ammonia in fungistasis of soils. *Phytopathol. Z.* 98, 310-319 (1980).

24- Zhao, J., Mei, Z. Zhang, X., Xue, C., Zhang, C., Ma, T., & Zhang, S. Suppression of Fusarium wilt of cucumber by ammonia gas fumigation via reduction of Fusarium population in the field. *Sci. Rep*. 7, 43103 (2017).

25- Brenneman, T. B., Csinos, A. S., & Phipps, P. M. Evaluation of ammonium bicarbonate for control of soilborne peanut pathogens. *Peanut Sci*. 17, 28-31 (1990).

26- Sun, L., Song, S., Fu, L., Deng, X., Wang, D., Liang, X., Li, R., & Shen, Q. Exploring a soil fumigation strategy based on ammonium bicarbonate to control Fusarium wilts of cucurbits. *Crop Prot.* 70, 53-60 (2015).

27- Rodriguez-Kabana, R., & King, P. S. Use of mixtures of urea and blackstrap molasses for control of root-knot nematodes in soil. *Nematropica*, 10, 38-44 (1980).

28- Huebner, R. A., Rodriguez-Kabana, R., & Patterson, R. M. Hemicellulosic waste and urea for control of plant parasitic nematodes: effect on soil enzyme activities. *Nematropica*, 13, 37-54 (1983).

29- Kim, W.K., & Patterson, P.H. Nutritional value of enzyme or sodium hydroxide- treated feathers from dead hens. Poult. Sci. 79, 528–534 (2000).

30- Jeong, J.H., Lee, O.M., Jeon, Y.D., Kim, J.D., Lee, N.R., Lee, C.Y., & Son, H.J. Production of keratinolytic enzyme by a newly isolated feather-degrading Stenotrophomonas maltophilia that produces plant growth-promoting activity. Process Biochem. 45, 1738–1745 (2010).

31- Tsavkelova, E., Oeser, B., Oren-Young, L., Israeli, M., Sasson, Y., Tudzynski, B., & Sharon, A. Identification and functional characterization of indole-3-acetamide mediated IAA biosynthesis in plant associated Fusarium species. Fungal Genet. Biol. 49, 48–57 (2012).

32- Allam, Y.A., & Nawwar, G.A.M. Facile synthesis of 3-spiroindolines. Heteroatom Chemistry. 13, 207-210 (2002).
33- Aly, M., El-Mageed, A., El Kafafy, A., & Nawwar, G. Synthesis and anti-aphid Aphis gossypii Glover activity of some new Quinoline derivatives. *J. Plant Prot. Res.* 51, 114-120 (2011).
34- Nawwar, G.A.M., Haggag, B.M., & Swellam, R.H. Synthesis and Molluscicidal Activity of New Derivatives of 1-(Hydroxy/substituted Phenyl)-3-arylpropenones. *Archiv der Pharmazie*. 326, 831-836 (1993).
35- Nawwar, G. A. Salicylamides containing amino acid or pyran moieties with molluscicidal activity. *Arch. Pharm*. 327, 201-205 (1994).
36- Nawwar, G.A.M., Zaher, K.S.A., Shaban, E., & El-Ebiary, N.M.A. Utilizing Semi-Natural Antibacterial Cellulose to Prepare Safe Azo Disperse Dyes and Their Application in Textile Printing. *Fibers Polym.* 21, 1293-1299 (2020).
37- Kamal, R., Kharbach, M., Imig, J.D. , Eljmeli, M., Doukkali, Z., NaceiriMrabti, H., Elmsellem, H., Bouklouze, A., Cherrah, Y., & Alaoui, K. Antioxidant activities, total polyphenolic compounds and HPLC/DAD/MS phenolic profile of argan oil derived from two different methods of extractions. JMES. 8, 1320-1327 (2017).
38- Alexander, M. Introduction to Soil Microbiology. 2nd Edition, John Wiley Eastern Limited, New Delhi, 467 (1977).
39- Ashoub, A. H., & Amara, M. T. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. *Am. J. Sci.* 6, 321-328 (2010).
40- Schmidt, J., & All, J. N. Attraction of Neoaplectana carpocapsae (Nematoda: Steinernematidae) to common excretory products of insects. *Environ. Entomol*. 8, 55-61 (1979).
41- Grewal, P. S., Gaugler, R., & Lewis, E. E. Host recognition behavior by entomopathogenic nematodes during contact with insect gut contents. *J. Parasitol*. 79, 495-503 (1993).
42- Ye , L., Han, Y. , Wang, X., Lu, X., Qi, X., & Yu, H. Recent progress in furfural production from hemicellulose and its derivatives: Conversion mechanism, catalytic system, solvent selection. *Mol. Catal*. 515, 111899 (2021).
43- El-Shahawy, T. A., El-Rokiek, K. G., Sharara, F. A., & Khalaf, K. A. New approach to use rice straw waste for weed control i. efficacy of rice straw extract against broad and narrow leaved weeds in cucumber (Cucumis sativa L.). *Int J Agric Biol* (Pakistan), 8, 262- 268 (2006).
44- Chen, X., Wang, X., Xue, Y., Zhang, T., Hu, J., Tsang, Y., Gao, M., Tapping the Bioactivity Potential of Residual Stream from Its Pretreatments May Be a Green Strategy for Low-Cost Bioconversion of Rice Straw. *Appl. Biochem. Biotechnol.* 186, 507–524 (2018).
45- Veverka, K., Štolcová, J., & Růžek, P. Sensitivity of fungi to urea, ammonium nitrate and their quimolar solution UAN. *Plant Prot. Sci*., 43, 157-164 (2007).
46- Singh, S., Kaur, I., & Kariyat, R. The multifunctional roles of polyphenols in plant-herbivore interactions. *Int. J. Mol. Sci.* 22, 1442 (2021).
47- Jabłońska–Trypuć, A., & Wiater, J. Protective effect of plant compounds in pesticides toxicity. *J. Environ. Health Sci. Eng*. 20, 1035-1045 (2022).
48- Liu, B., Li, N., Chen, F., Zhang, J., Sun, X., Xu, L., & Fang, F. Review on the release mechanism and debittering technology of bitter peptides from protein hydrolysates. *CRFSFS*. 21, 5153-5170 (2022).
49- Akbarmehr, A., Peighambardoust, S. H., Ghanbarzadeh, B., & Sarabandi, K. Physicochemical, antioxidant, antimicrobial, and in vitro cytotoxic activities of corn pollen protein hydrolysates obtained by different peptidases. *Food Sci. Nutr*. 11, 2403-2417 (2023).
50- Goodman, B. A. Utilization of waste straw and husks from rice production: A review. *J.Bioresour.Bioprod.* 5, 143-162 (2020).
51- Zhang, X., Gao, D., Luo, W., Xiao, N., Xiao, G., Li, Z., Liu, C. Hemicelluloses-based sprayable and biodegradable pesticide mulch films for Chinese cabbage growth. *Int. J. Biol. Macromol*. 225, 1350-1360 (2023).