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The efficacy of fish and cow vermicompost in managing Rhizoctonia damping off on cucumber

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ABSTRACT

Two different vermicomposts were prepared from cow dung (CVC) and fish sludge (FVC) using a mixture of three species of earthworms *i.e. Eisenia fetida, Lumbricus rubellus* and *Perionyx excavates.* The produced vermicomposts were evaluated for their ability to control pre- and post- emergence damping-off disease on cucumber plants caused by *Rhizoctonia solani*. Pot experiments were conducted in two seasons (2022/2023-2023/2024). Both CVC and FVC significantly reduced pre- and post- emergence damping-off and enhanced plant growth as reflected in roots and shoots dry weights. CVC was the most potent in inducing phenolic compounds in treated plants compared to other treatments. CVC surpassed FVC in its ability to inhibit DPPH levels, but both showed a significant effect. Additionally, both CVC and FVC treatments led to a significant increase in peroxidase and catalase activities compared to the untreated control. The expression of defense-related marker genes, *i.e.* SA (NPR1 and PR1), JA (LOX1 and PR3), and Phenylalanine ammonia-lyase (PAL) was significantly upregulated in most treatments involving vermicomposts. NPR1 was significantly upregulated in plants treated with CVC and challenged with *R. solani*. PR1 was upregulated in plants treated with either CVC or FVC and challenged with *R. solani*. FVC was more effective at upregulating LOX1 expression, while both CVC and FVC showed significant and very similar upregulation of PR3. Interestingly, PAL expression was not significantly upregulated by FVC, and it was even downregulated by CVC alone compared to the untreated control.

Keywords: cucumber, vermicompost, Rhizoctonia damping off, defence enzyme activities, defence related genes

INTRODUCTION

Over the past few decades, considerable research efforts have been devoted to developing plant disease control strategies that are both environmentally friendly and safe for humans and other living organisms. One well-established approach involves converting organic wastes into compost materials, a practice that has been adopted for decades and offers undeniable environmental benefits while producing rich, useful products for agricultural purposes. Among these methods, vermicomposting where earthworms degrade organic materials under aerobic conditions has gained significant attention. This process not only relies on bacteria that break down organic waste and make it more accessible to worms for further decomposition, but also benefits from the internal bacteria living inside the worms' gut, which play a crucial role in breaking down organic compounds and facilitating the composting process (Adhikary, 2012; Chattopadhyay, 2012; Vuković, 2021). The benefits of using vermicompost are numerous and depend largely on the type of the organic waste being composted. Earthworms can degrade various types of organic and biodegradable wastes, including livestock wastes, agricultural wastes, food wastes, and organic household wastes (Chattopadhyay, 2012). Accordingly, the characteristics and effects of the resulting vermicomposted material depend on the origin of the degraded waste and the composting method used (Saba *et al.*, 2023).

Applying vermicompost to the soil improves soil aeration and structure, enhances fertility, and stimulates beneficial soil microorganisms (Vuković *et al.*, 2021). Moreover, the role of vermicompost in controlling plant diseases has been extensively documented. A study has revealed that root rot and damping-off diseases on different crops were significantly reduced when vermicompost was mixed with the soil before planting (Pathma and Sakthivel, 2012; Basco *et al.*, 2017; You *et al.*, 2019; Yatoo *et al.*, 2021). You *et al.*, (2019) reported a significant reduction in damping-off on cucumber when nursery soil was mixed with vermicompost bamboo powder. In addition, a significant decrease in infection was recorded in tomato seedlings infected with

Phytophthora nicotianae after they were treated with vermicompost produced from animal manure (Szczech and Somlinska, 2001).

Cucumber, (*Cucumis sativus* L.), s the third most-produced vegetable crop globally, preceded only by tomato and onion (http://www.fao.org/faostat/en/#data/QC). At different stages of their growth, cucumber plants are prone to various infections that can be detrimental to the plants and lead to significant yield reductions, especially if these diseases affect them during their early stages (Bondarenko *et al.,* 2021). The first stage of plant development is undoubtedly the most vulnerable, making damping-off a critical threat that can result in complete crop failure. *Rhizoctonia solani*, can attack cucumber plants during seedling stage, causing damping-off and its effect can extend to more advanced growth stages causing damage to root tissues and significantly impacting plant growth (Hassan *et al.,* 2021; Saeed and Othman, 2023). Due to its wide host range and the ability to survive for long periods in the form of sclerotia, diseases caused by *R. solani* are considered quite challenging to control. Therefore, the early application of control measures in addition to following the recommended agriculture practices

The purpose of this study was to evaluate two types of vermicompost, developed from cow dung and fish sludge, for their efficacy in controlling pre-emergence and post-emergence damping-off in cucumber plants caused by *R. solani* and to study the changes in the expression of genes related to pathogenesis and how the applied vermicompost affects their expression patterns.

MATERIALS AND METHODS

Study location:

This study was conducted in the greenhouses at Plant Pathology Research Institute, ARC and Laboratory for Aquaculture Research (CLAR), during two consecutive seasons, 2022-2023 and 2023-2024.

Vermicompost production*:*

Two different vermicompost were prepared using a mixture of three species of earthworm i.e. *Eisenia fetida, Lumbricus rubellus* and *Perionyx excavates,* according to (Moustafa *et al.,* 2023).

All parameters measured and based on dry weight. The analysis was conducted by Soils, Water and Environment Research Institute, Agriculture Research Center, Egypt.

Preparation of different vermicompost materials:

Cow dung (CVC) processing:

Fresh cow dung was obtained from some private cow farms located near to the Central Lab for Aquaculture Research (CLAR), Sharkia, Egypt. It was moistened to 60-70%, an initial moisture content of approximately 50%, before being processed with earthworms.

Fish sludge (FVC) collection and preparation*:*

Fish sludge was collected from the concrete ponds of Nile tilapia (*Oreochromis niloticus*) broodstock and fry, at Nile tilapia hatchery belonging to CLAR; during fry harvesting from the broodstock ponds as well as from fry rearing ponds. The produced fish, with a moisture content of 96.5% and dried solid content of 3.5% was collected in barrels and then spread out in a thin layer on a cement floor and dried for 14 days for safe storage and future use.

Earthworm inoculation and Vermicompost production:

Both fresh cow dung and dried fish sludge were prepared individually and moistened to 60-70% in Styrofoam boxes (60×40×30 cm). After 24 hrs, three species of earthworm (*E. fetida, P. excavatus and L. rubellus*) were added to the media at a rate of 50 g of worm per 1000 g media for 8 weeks. The boxes were checked weekly and re-moistened and mixed until the vermicompost matured. All boxes were kept indoors, and the temperature was maintained between 18-25°C during the vermicompost maturation period. At harvest time, vermicompost was checked manually on a white plastic surface and all adult, and pre-adult earthworms, were collected. Then the vermicompost then was returned to the boxes for one more month. Subsequently, the vermicompost was re-checked again and all hatched earthworms were collected. The harvested vermicompost was packed in plastic bags and samples of the two vermicompost types (CVC and FVC) delivered to laboratories to be analyzed.

Vermicomposts characterization:

Some physical, chemical, and biological characteristics of the produced CVC and FVC were determined. First, the vermicompost, rested for 3 months after harvest, was sieved through a 2-mm screen and dried at room temperature before all analyses. Organic matter content, total nitrogen, C:N ratio, and cation exchange capacity were determined according to the procedures described by (Kacar, 2003). Specifically, water content was measured by drying 5 g of fresh vermicompost at 65 °C; EC and pH were determined using an EC and pH meter in 1:5 (v:v) and 1:2.5 (w:v) vermicompost-to-1 N KCl mixtures, respectively, after continuous shaking for 1

h. Total N was analyzed by the Kjeldahl method, the C:N ratio by the combustion method at 550 °C, and cation exchange capacity by the ammonium acetate procedure, in which dried vermicompost was saturated with 1 N sodium acetate (pH 7) and sodium was displaced with 1 M ammonium acetate before flame photometer reading. Micronutrient analysis of a water extract of the vermicompost (1:2, v/v, with distilled water) was carried out with an atomic absorption spectrophotometer (Model Varian Vista).

The number of bacteria and actinomycetes in vermicompost were determined by plate count technique using selective media as described by (Szczech, 1999). The results were expressed as number of colony-forming units (cfu) per 1 g of vermicompost dried at 105^{o}C.

Efficacy of vermicoposts on suppressing *R. solani* damping-off disease on cucumber:

Pot experiments were conducted in two consecutive seasons (2022-2023 and 2023-2024) to study the impact of two types of vermicomposts (cow vermicompost and fish vermicompost) in controlling cucumber damping-off caused by *R. solani*.

R. solani* inocula*:

R. solani strain was obtained from the culture collection of Mycology Research and Disease Survey Dept. Plant Pathology Research Institute, ARC. The strain was cultured on Potato Dextrose Agar (PDA) autoclaved on Petri plates (9cm) and incubated at 25 ± 2°C for 5 days. To enrich the fungus, agar discs were transferred from the PDA plates culture to glass bottles containing autoclaved cornmeal sand medium (75 g grinded corn meal, 25 g fine washed sand and 50 ml tab water). Bottles were incubated at 25 ± 2°C for 7-10 days. Pot experiment was carried out using 25 cm pots filled with sterilized sandy loam soil mixture. Fungal inoculum was mixed with the soil in each pot at the rate of 3% (w/w). Pots used as control were left without inoculum. Pots were distributed in a completely randomized design. Three cucumber seedlings (Cevher cv.) were sown in each pot, and three replicates were used for each treatment.

This experiment comprised of seven groups of treatments as follows:

Group 1: cucumber seedling planted in pots consisting of *R. solani* and treated with recommended dose of the fungicide Rhizolex.

Group 2: cucumber seedlings planted in pots consisting of *R. solani* and treated with Cow Dung vermicompost.

Group 3: cucumber seedlings planted in pots consisting of *R. solani* and treated with Fish Sludge vermicompost.

Group 4: cucumber seedlings planted in uninoculated pots (control).

Group 5: cucumber seedlings planted in pots consisting of *R. solani*.

Group 6: cucumber seedlings planted in pots and treated with Cow Dung vermicompost (CVC).

Groups7: cucumber seedlings planted in pots and treated with Fish Sludge vermicompost (FVC).

Disease assessment*:*

Percentages of pre- and post-emergence damping-off as well as healthy survival plants in each treatment were determined after 15, 30 and 45 days of planting, respectively. Root rot was recorded according to (El-Helaly *et al*., 1970).

Plants after harvested the second season were subjected to further measurements of root and shoot dry weights, plant length and leaf number. In addition, samples of these plants were also used for further biochemical analysis.

Biochemical studies:

Cucumber plants were collected after 45 days of planting to determine the total phenolic compounds and the antioxidant as well as peroxidase and catalase activities.

Preparation of cucumber root extracts:

Treated cucumber roots were collected to evaluate total phenol content and antioxidant activity. After washing with tap water, the roots were freeze-dried using an at −40 °C for 48 hours. The resulting dry material was ground, stored at −40 °C, and later macerated in methanol for two days. The extracts were filtered, concentrated under vacuum at 50 °C, and re-suspended in methanol at 1 mg/mL for subsequent analyses.

Determination of total phenolic contents.

Total phenolic content in cucumber root extract was measured using the Folin-Ciocalteu method (Mekawi *et al.,* 2019), with absorbance read at 650 nm. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

Determination of antioxidant activity:

DPPH radical scavenging assay:

Scavenging activity of different extract against DPPH radicals was assessed according to the method of (Mekawi *et al.*, 2019) with a slight modification. One milliliter of DPPH radical solution (0.1 mM) in methanol

was added to 3 ml of methanolic of root at different concentrations (10–150 µg/mL). Then the absorbance was determined at 517 nm against blank (methanol pure). The blend was shaken violently and left at room temperature for 30 min. in the dark. The butylated 4-Hydroxyl toluene (BHT) was used as a positive control; and negative control contained the entire reaction reagent except the extracts. The DPPH scavenging percentage effect (%) was calculated utilizing the following equation:

DPPH_ scavenging effect (Inhibition %) = [(Ac - A_S/Ac)] x 100

Where: Ac was the absorbance of the control reaction and as was the absorbance in the presence of the plant methanolic extract.

Tested plant extracts concentration and the ordinate illustrate the average percent of scavenging capacity (Excel program).

Determination of antioxidant enzymes:

Peroxidase (EC 1.11.1.7) was assayed following the method described by Ashry and Mohamed (2012). The colour intensity was read at 430 nm, and the enzyme activity was expressed as the change in the optical density/gram fresh weight/hour.

Catalase (EC 1.11.1.6) was assayed following the method of (Ashry and Mohamed, 2012). The colour intensity was read at 240 nm. Catalase activity was expressed as µmole H2O2 destroyed/gram fresh weight/hour.

Total RNA isolation and cDNA synthesis:

The total RNA was extracted from frozen cucumber plants' leaves using TRIzol reagent following the recommended procedures (ThermoFisher, USA). The RNA was then treated with RNase-free DNase I (ThermoFisher, USA) to eliminate DNA contamination. Subsequently, RNA presence and integrity were assessed via 1.0% agarose gel electrophoresis and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, MA, USA). For cDNA synthesis, 1.2 µg of total RNA was utilized with the M-MLV Reverse Transcription Kit (Promega Corporation, United States). The resulting cDNA was diluted to a 1:10 ratio before proceeding to quantitative PCR (qPCR) analysis. The qPCR-specific primers were designed using the PrimerQuest Tool available at (https://www.idtdna.com/PrimerQuest/Home/Index) (Table 1).

Table 1. The qPCR-specific primers (Pu, *et al.,* 2014; Mohamed, *et al.,* 2024)

Gene	Sequence
CS_NPR1-F	TTACTGATAAGGGCAAGAAGGCC
CS_NPR1-R	AAAGTTCACAAAGAGCAGGATGG
CS_PR1-F	GTGCCTTGTGATGAAGTAGG
CS_PR1-R	CCACACTAGAGGAGGTTGAT
CS_PAL1-F	GCAGTGCCACTTATCCATTA
CS_PAL1-R	GGCGTTCTTCTCATCTTCTC
CS_PR3-F	TTCCGACATCGAAGCTTTAC
CS_PR3-R	GGTTGGAAGAACTTGAGAAATAAG
CS_Actin-F	GGCCGTTCTATCACTGTATG
CS_Actin-R	GAGCATAACCCTCGTAGATTG

Quantitative PCR (qPCR) analysis:

To validate the expression levels of all samples, qPCR analysis was conducted using Solg™ 2X Real-Time PCR Smart Mix (SYBR Green Mix; SolGent Co., Korea) on an Agilent Stratagene Mx3005p real-time PCR detection system following the manufacturer's instructions. Each reaction consisted of a total volume of 20 µL, including 1 µL of diluted cDNA, 10µL of 2 × SYBR Green PCR Master Mix, and 0.3 µL of each forward and reverse primer (10 µm). Amplification was carried out with the following program: an initial denaturation at 95°C for 15 min, followed by 40 cycles at 95°C for 3 s and 60°C for 40 s. To ensure the specificity of the qPCR products, a melting curve analysis was performed within the temperature range of 65°C to 95°C. The Actin gene served as the internal reference gene for normalization. All reactions were performed in triplicate, and the relative expression levels were determined using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

Statistical analysis*:*

The experiment was designed as a completely randomized design (CRD). Statistical analysis was conducted using SPSS v23.0 (Chicago, IL, USA) through a one-way analysis of variance (ANOVA). Duncan's multiple range test was employed to assess significant differences between means at a threshold of $P<0.05$. In addition, correlation analyses between defense-related gene expression levels and the number of survived cucumber plants were carried out using the same statistical software (Gomez and Gomez, 1984).

RESULTS

Characterization of CVC and FVC:

The chemical, physical and biological characterizations of both types of vermicompost were studied. Data presented in (Table 2) showed that some contents, *i.e.* total nitrogen, ammonia, nitrate, organic carbon, phosphorus and potassium were higher in CVC compared to the FVC.

Table 2. Physical, chemical and biological parameters of CVC and FVC.

Item	Moisture (%)	pH	EC (ds/m)	Total (NH3+NH4) (ppm)	Total (NO3) (ppm)	Total Nitrogen (%)	Organic matter (%)	Organic C (%)	Ash (%)	C:N Ratio	Phosphorus (%)	Potassium (%)	Total bacteria count (Cell/g)	Total actinomycetes (Cell/g)
CVC[$]	78	7.61	0.48	46	18	2.01	51.55	29.9	48.45	15:1	1.42	0.67	$8*10^6$	$18*10^5$
FVC[$]	67	7.82	0.43	37	18	0.83	27.98	16.23	72.02	20:1	0.49	0.49	$7*10^6$	$10*10^5$

CVC: Cow vermicompost FVC: Fish vermicompost:

However, the ash content in FVC was found to be higher (72.02%) compared to CVC (48%). Additionally, the total counts of actinomycetes and total bacteria were higher in the case of CVC than in the case of FVC, with an observable difference in the latter.

Efficacy of CVC and FVC in suppressing the infection with *R. solani* in cucumber:

A pot experiment was conducted to study the efficacy of using CVC and FVC in suppressing damping-off and root rot caused by *R. solani* on cucumber plants. Data revealed a significant effect of both types of vermicompost in suppressing both pre- and post-emergence damping off. However, CVC was more effective in suppressing pre-emergence damping-off in both seasons while in the post-emergence stage both types of composts were equally effective in suppressing the damping off in both seasons.

Table 3. The Effect of CVC and FVC on pre- and post-emergence damping-off of cucumber plants (Cevher cv) caused by *R. solani* for two successive seasons (2022/2023-2023/2024).

Treatments	First season (2022/2023)					Second season (2023/2024)				
	Pre-emergence	Post-emergence	Survival plants	*Treatment efficiency of survival plants%	**vermicompost efficiency to fungicide efficacy %	Pre-emergence	Post-emergence	Survival plants	*Treatment efficiency of survival plants%	**vermicompost efficiency to fungicide efficacy %
CVC + *R. solani*	22.22 [c]	11.11 [b]	66.67 [c]	199.86	79.98	11.11 b	22.22 [b]	66.67 [c]	99.64	59.94
FVC+ *R. solani*	33.33 [b]	11.11 [b]	55.56 [d]	149.93	60.00	22.22 a	22.22 [b]	55.56 [d]	66.36	39.92
CVC control	0.00 [e]	0.00[c]	100.0 [a]	349.84	140.00	0.00 [e]	0.00[c]	100.0 [a]	199.49	120.02
FVC control	0.00 [e]	0.00[c]	100.0 [a]	349.84	140.00	0.00 [e]	0.00[c]	100.0 [a]	199.49	120.02
R. solani +Fungicide	11.11 [d]	11.11 [b]	0.00[c]	249.88	100	11.11[b]	0.0 [d]	88.89 [b]	166.21	100
R. solani control	44.44 [a]	33.33 [a]	44.44[a]	0.00	0.00	22.22 [a]	44.44 [a]	33.34 [e]	0.00	0.00
Control	0.00 [e]	0.00[c]	100.0 [a]	349.84	140.00	0.00 [e]	0.00[c]	100.0 [a]	199.49	120.02
L.S.D. at (5%)	0.257	0.0795	0.1591	---	---	0.0562	0.0562	0.2977	----	---

Regarding the survived cucumber plants at the end of the season, data showed that CVC gave significant better results in both seasons compared to FVC (Table 3). It is also noticeable that there was a slight negative effect of both studied vermicompost on plant growth during pre and post emergence stages in addition to some dead plants that were recorded at the end of the experiment. Plant treated solely with CVC or FVC were negatively affected compared to the control. In the second season a 11.11% damping-off was recorded when plants were treated with either type of composts.

Table (4) demonstrates the significant impact of vermicompost on cucumber growth, particularly on the number of leaves and the plant height. Plants treated with either CVC or FVC clearly showed improvement in different growth characteristics compared to control treatments. The CVC + *R. solani* treatment showed the highest plant length (64.6 cm) and a relative high leaves number (17.55), while the FVC control and CVC control treatments also recorded substantial growth metrics, highlighting the standalone efficacy of vermicompost. Data clearly shows that using the Rhizolex fungicide significantly improved plant growth compared to the *R. solani* sole treatment (36.3 cm plant length and 6.6 leaves) but vermicompost treatments significantly outperformed the chemical fungicide in improving plant growth.

Table 4. Effect of CVC and FVC on plant length and leaves number of cucumber plants (Cevher cv)

Treatments	Plant length			Leaf number		
	Mean plant length	*Treatment efficiency %	**vermicompost efficiency to fungicide efficacy %	Mean leaf number	*Treatment efficiency %	**vermicompost efficiency to fungicide efficacy %
Cow + *R. solani*	64.6 [a]	192.03	316.82	17.55 [b]	416.66	500.01
Fish + *R. solani*	62.0 [a]	192.03	316.82	16.33 [b]	380.55	456.67
Cow control	66.0 [a]	185.84	306.61	20.00 [a]	455.55	546.68
Fish control	66.0 [a]	174.33	287.62	18.00 [b]	400.00	480.01
Rhizolex + *R. solani*	36.3 [c]	60.61	100.00	6.60 [c]	83.33	100.00
R. solani control	22.6 [d]	0.00	0.00	3.60 [d]	0.00	0.00
Control	43.3 [b]	91.59	151.11	16.30 [b]	352.77	423.34
L.S.D. at (5%)	5.23	---	---	1.88	----	----

* % Treatment efficiency = ((Control-treatment)/Control) X 100

** % vermicompost efficiency to fungicide efficacy = (treatment efficiency / fungicide efficacy) X 100

Table 5. Effect of CVC and FVC on shoot and root dry weight of cucumber plants (Cevher cv**)**

Treatments	Shoot dry weight			Root dry weight		
	Weight(g)	*Treatment efficiency %	**vermicompost efficiency to fungicide efficacy %	Weight (g)	*Treatment efficiency %	**vermicompost efficiency to fungicide efficacy %
CVC + *R. solani*	45.3 [a]	642.62	198.98	5.0[b]	400.00	307.69
FVC+ *R. solani*	34.4[b]	463.93	143.65	4.9[b]	390.00	300.00
CVCcontrol	44.7[a]	632.73	195.93	5.8[a]	480.00	369.23
FVC control	28.4[c]	365.57	113.19	5.3[ab]	430.00	330.76
Rhizolex + *R. solani*	25.8[cd]	322.95	100.00	2.3[c]	130.00	100.00
R. solani control	6.1[e]	0.00	0.00	1.0[d]	0.00	0.00
Control without treatment	23.7[d]	89.33	89.33	2.4[c]	140	107.69
L.S.D. at (5%)	2.94	---	---	0.665	---	---

* % Treatment efficiency = ((Control-treatment)/Control) X 100

** % vermicompost efficiency to fungicide efficacy = (treatment efficiency / fungicide efficacy) X 100

Cow+ R =CVC+ *R. solani,* Fish+R= FVC+ *R. solani* , Cow control= CVC control, Fish control= FVC

The effect of FVC and CVC in plant dry weight was assessed. Data presented in (Table 5) indicates that both kinds of vermicompost positively enhanced plant growth reflected in root and shoot dry weight. Roots dry weight was significantly higher in all treatments with vermicompost compared to untreated control or fungicide treatment. No significant differences in roots dry weight were recorded among treatments involving vermicompost. On the other hand, the effect of vermicompost on shoots was more discernible as both treatments clearly improved shoots growth compared to untreated control plants. In general, CVC was more effective in promoting shoot growth compared to FVC.

Biochemical studies:

The levels of phenolics, antioxidants (DPPH % inhibition), peroxidase and catalase activities were assessed across different treatments to evaluate the plant's defence response against *R.a solani* and to determine how the applied vermicomposts affect this response.

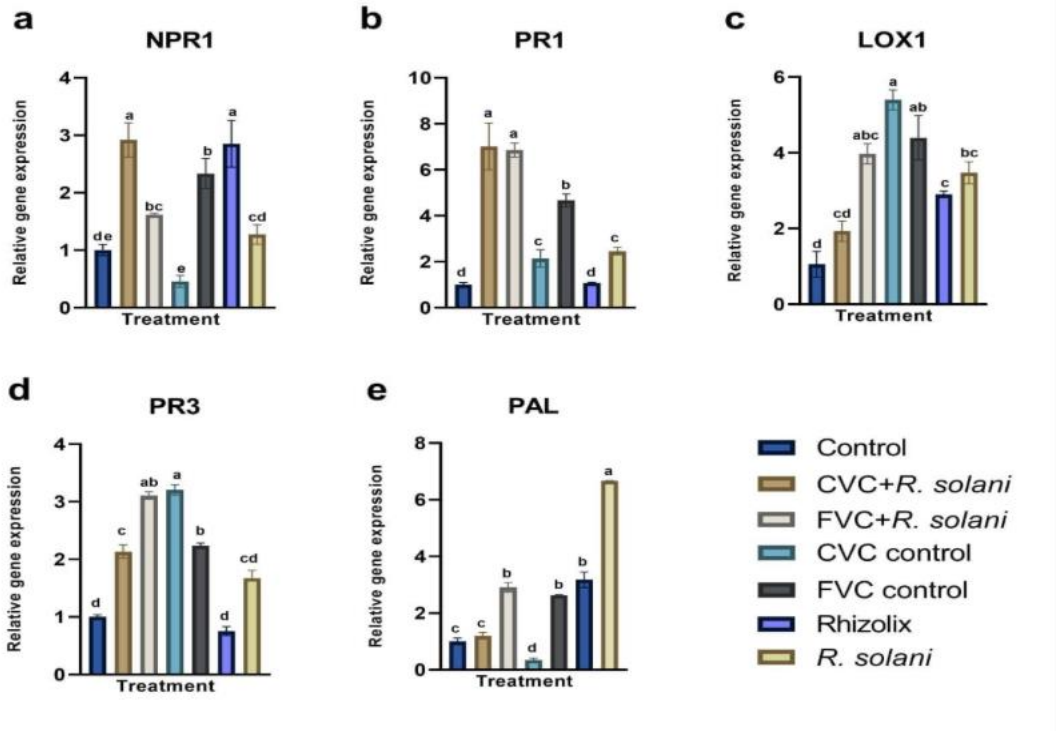
Table 6. Effect of CVC and FVC vermicompost on defenceenzyme activities, total phenols and antioxidants (DPPH) in cucumber plants (Cevher cv).

Treatments	Total phenol (mg GAE/g DW	Antioxidants DPPH radical scavenging activity	Peroxidase (activity/g fresh weight/h)	Catalase (activity µM H2O2/ g fresh weight/h)
CVC + *R. solani*	248.3 [a]	61.2 [a]	181.5 [a]	117.6 [a]
FCV + *R. solani*	230.1 [b]	56.0 [b]	165.8 [b]	112.4 [c]
CVC control	201.3 [c]	52.2 [cd]	161.2 [c]	108.2 [d]
FCV control	199.1 [c]	50.1 [d]	146.3 [d]	98.3 [e]
Rhizolex + *R. solani*	249.0 [a]	53.0 [c]	168.0 [b]	115.5 [b]
***R. solani* control**	108.0 [d]	22.0[e]	54.1[e]	31.7 [f]
Control	78.0 [e]	12.3 [f]	30.1 [f]	21.3 [g]
LSD at (5%)	2.3	1.2	2.1	0.6

Data presented in (Table 6) indicates that *R. solani* triggered cucumber plants to produce phenolic compounds (108.0 µg/g). The highest phenolic content was recorded in Rhizolex + *R. solani* treatment (249.0 µg/g) and CVC + *R. solani* (248.3 µg/g) treatment. FVC + *R. solani* treatment had slightly lower phenolic compounds (230.1 µg/g), followed by single vermicompost treatments (199.1 & 201.3 µg/g). Data also demonstrates that DPPH% inhibition was significantly influenced by both *R. solani* and vermicompost treatments. The application of CVC significantly increased DPPH inhibition level to 61.2%, the highest among all treatments, followed closely by FVC + *R. solani* treatment (56.0%) and the Rhizolex + *R. solani* treatment (53.0%). Regarding peroxidase and catalase, data showed clear relative increase on their concentrations in all treatments in comparison to untreated control plants. However, CVC and FVC had more potent effect in stimulating peroxidase and catalase production in treated plants.

The effect of CVC and FVC application on the expression levels of defence related genes in cucumber plants:

A qPCR assay was conducted to study how using the CVC and FVC in suppressing infection with *R. solani* could affect the expression of defense-related marker genes, *i.e.* SA (NPR1 and PR1), JA (LOX1 and PR3), and Phenylalanine ammonia-lyase (PAL). Cucumber plant leaves from different treatments were collected at the end of 50 days to perform the qPCR assay.



Cow+ R =CVC+ *R. solani,* Fish+R= FVC+ *R. solani* , Cow control= CVC control, Fish control= FVC

Fig. 1. Relative expression profiles of five defense-related genes in cucumber plants (Cevher cv.) treated with CVC and FVC using a real-time PCR assay.

The expression profiles were estimated after normalization with ubiquitin-conjugating protein house-keeping gene. Data bars are the mean standard deviation of ΔCt expression:

The data illustrated in (Fig. 1) clearly shows that the Rhizolex and CVC+ *R. solani* treatments exhibited a significant upregulation of *NPR1* expression levels, by 2.9-fold. The Fish control treatment exhibited a moderate *NPR1* expression level by 2.33-fold, while the FVC+ *R. solani* and *R. solani* treatments showed expression levels of 1.62- and 1.27-fold, respectively compared to the control. On the other hand, there was no significant difference in the expression level of NPR1 under CVC control treatment compared to the control (Fig. 1 a). The expression level of PR1 was significantly upregulated by 7-fold under CVC+ *R. solani* and FVC+ *R. solani* treatments, and by 4.6-fold under the FVC control treatment, compared to the control, *R. solani*, and Rhizolix treatments (Fig. 1 b). Regarding the LOX1 gene, FVC control and FVC+ *R. solani* treatments resulted in a significant upregulation in gene expression by 4-fold compared to the control. Although CVC control treatment also significantly upregulated LOX1 by 5.4-fold, its expression level was only slightly increased by 2-fold under CVC+R and did not show a significant change compared to the control (Fig. 1 c).

The expression level of PR3 was significantly upregulated by 3-fold and 2-fold under FVC+*R. solani* and FVC control treatments, respectively, compared to all other treatments. Similarly, both CVC control and CVC+ *R. solani* treatments exhibited significant upregulation of PR3 by 3- and 2-fold, respectively, compared to the control (Fig. 1d). The expression of PAL was significantly upregulated by 6.7-fold under *R. solani* treatment compared to the control. However, no significant differences in PAL expression levels were observed under FVC+ *R. solani*, FVC control, and Rhizolix treatments with fold changes ranging from 2.6 to 3. Notably, PAL expression was significantly downregulated by 0.34-fold under the CVC control treatment compared to both CVC+ *R. solani* and the control (Fig. 1e).

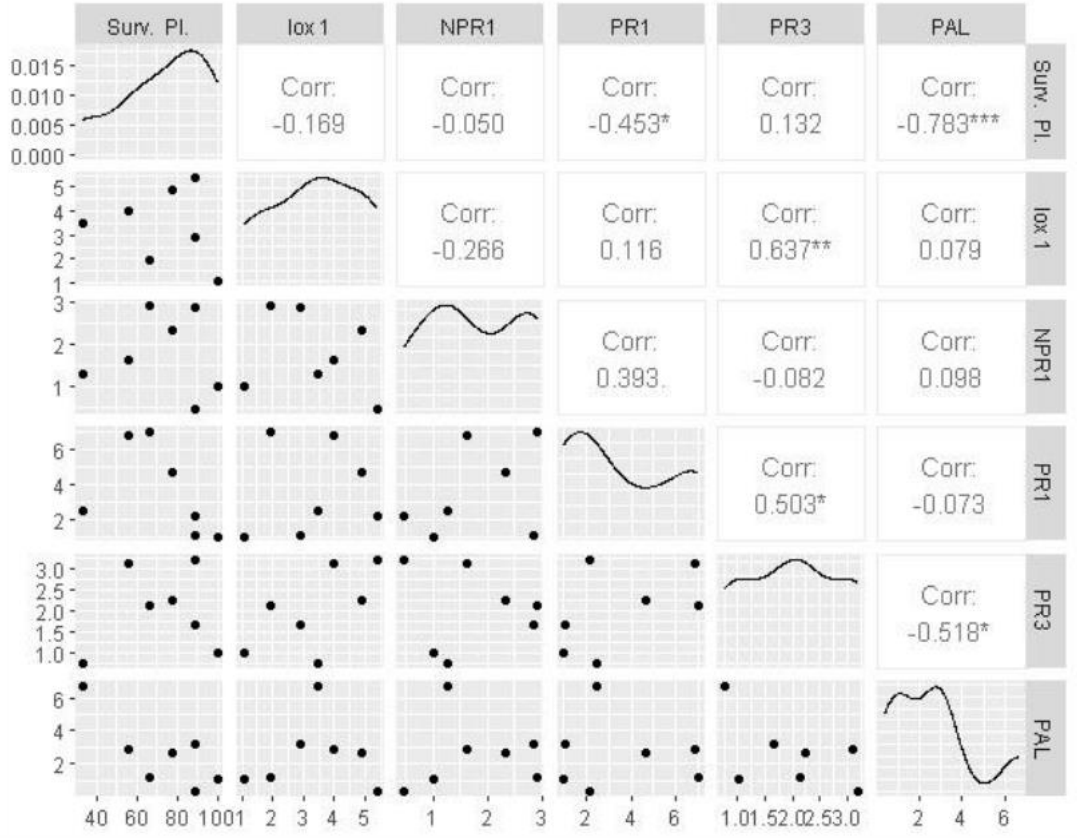


Fig. 2. The correlation between the expressions in defense-related genes and the number of survived cucumber plants

Expression of the PR1 gene showed a tendency to be associated with reduced plant survival. In contrast, the expression level of lox1 was positively correlated with that of PR3 (Fig. 2). Notably, PR1 and PR3 expression levels also appeared to co-vary, suggesting potential co-regulation of these defense-related genes. Overall, these findings highlight relationships between gene expression and cucumber plant survival after infection, indicating that genes such as PAL and PR1 are associated with reduced survival, whereas lox1 and PR3 may act in concert or be co-regulated to support plant defense.

DISCUSSION

The innate ability of earthworms to digest organic matter and convert it into a nutrient-rich compound known as vermicompost has been widely utilized. The Vermicompost provide a means by which plant disease control can be achieved while avoiding the extensive application of chemical pesticides (Edwards *et al.,* 2004). Moreover, vermicompost characterized by its high nutrient content and the presence of plant growth promoters as auxins, gibberellins, cytokinins, and hormones that stimulate plant growth. Along with beneficial microbes, the contributes to the suppression of diseases associated with soil-borne pathogens (Yatoo *et al.,* 2021; Rehman *et al.,* 2023). The present study aimed assessing two types of vermicompost for their potential suppressive effect on cucumber damping-off diseases. Both the source of organic compounds and the technology used in vermicompost production affect the characteristics of the produced compost. In the present study, chemical and biological characteristics of the cow vermicompost (CVC) and fish vermicompost (FVC) revealed distinctive differences between them. Data clearly showed that the ammonia content was higher in CVC than in FVC, whereas FVC had a higher nitrate content than CVC. Overall, the total N content was higher in CVC compared to FVC. The high N indicates a good fertilizing potential but if this in the form of ammonia this

suggests less maturity of the compost, slower release of N and a potential phytotoxicity of the compost (Saba *et al.,* 2023; Lončarić *et al.,* 2024). Therefore, CVC is a rich source of N but the accessibility for plants would probably be slower due to the immaturity. In other words, the composting duration was insufficient for CVC to be mature and safe for treated plants.

In addition, CVC showed higher content of organic matter, P and K compared to FVC. Our results also showed richness in bacterial and actinomycetes populations in both types of vermicompost. The beneficial bacterial and actinomycetes communities present in the vermicompost is considered an important element of the compost quality). Bacterial groups such as Azotobacter, the Nitrogen fixing bacteria, and phosphate solubilizing bacteria are commonly found in vermicomposts and they play a vital role in improving plant growth (Adhikary, 2012; Liu *et al.,* 2021; Vukovi´c, *et al.,* 2021). Furthermore, some plant growth promoting compounds (hormones) such as Indole-3-acetic acid (IAA), Gibberellins and cytokinins, in addition to some antibiotics are produced by a large group of actinomycetes (Sousa and Olivares, 2016; Khan *et al.,* 2023). In the present study a pot experiment was conducted to investigate the efficacy of CVC and FVC on suppressing pre/post emergence damping off caused by *R. solani* on cucumber plants. Data clearly showed a significant effect of both vermicompost in suppressing the effect on both stages (pre and post emergence) compared to untreated control plants (Al Masoudi and Muhammadawi, 2025). Numerous studies reported a suppressive effect of vermicompost against multiple soil-born plant diseases (Manandhar and Yami, 2008; Basco *et al.,* 2017; Liu *et al.,* 2021).

The suppressive effect of vermicompost to plant diseases is suggested to be largely due to the stimulation of the antagonistic bacteria and beneficial microbial communities present in the soil. Moreover, the microbial content of these composted organic compounds itself also contributes to this kind of suppressive effects against plant disease (Szczech, 1999). As mentioned above, the two kinds of vermicompost under investigation showed richness in bacterial and actinomycetes populations. In addition, composts are great sources of minerals and plant supplements. This was clear from our findings since both CVC and FVC characterization showed high concentrations in different elements such as N, P, K and C that are considered essential for plant growth and largely contribute to plant vigour. In this regard, our data also showed a significant increase in both root and shoot dry weight compared to the untreated control plants (Ma *et al.,* 2022). Ironically, data showed a slight suppressive effect of both FVC and CVC since some pre/post emergence damping offs were recorded in vermicompost single treatments. These findings emphasize on a potential toxicity of both cow and fish vermicompost under investigation. The phytotoxicity of composts were previously investigated and was related to immaturity of the composts being applied (Lončarić *et al.,* 2024).

Biochemical studies were conducted to investigate how plants could respond in a deeper level to vermicompost treatments and how this is connected to plant diseases resistance. The assessed defence parameters were phenolics, antioxidants (DPPH % inhibition), peroxidase and catalase levels. The results revealed that all treatments involving vermicomposts under investigation significantly enhanced all measured biochemical defence parameters compared to other treatments. It was clear that both vermicomposts enhanced the production of phenolic compounds. The highest concentration of total phenols was recorded in CVC treatment with *R. solani* which indicates a more potent trigger for defence response in plants compared to FVC. The same pattern was observed for DPPH inhibition levels as all treatments, surpassed by CVC, enhanced antioxidant defence system (Talaat and Abdel-Salam, 2024). The increase in phenolic compounds and antioxidant activity suggests that compost amendments may prime the plant's secondary metabolism and enhance its ability to scavenge reactive oxygen species (ROS), a common stress response mechanism during pathogen attack (Bennett and Wallsgrove, 1994; Vukovi´c *et al.,* 2021).

Similarly, the levels of peroxidase and catalase were increased in vermicompost treated plants. These enzymes play a critical role in the plant defence system. Both catalase and peroxidase coordinate to enhance plant resistance under biotic stress. They play essential roles in mitigating oxidative stress in plant cells by detoxifying reactive oxygen species (ROS) generated during biotic stress (Saddique *et al.,* 2018; Appu *et al.,* 2021; Tarfeen *et al.,* 2022). To elucidate the molecular basis underlying the effect of vermicompost on suppressing damping-off disease on cucumber, the expression of some key defense-related genes *i.e.* NPR1, PR1, LOX1, PR3, and PAL was studied. The results revealed a consistent and significant up-regulation of these genes in plants treated with both kinds of vermicompost and challenged with *R. solani* infection. Regarding NPR1 and PR1, plants treated with CVC and *R. solani* showed the highest expression levels, while LOX1 and PR3 showed a higher up-regulation in plants treated with both FVC and *R. solnai* compared to CVC treatment. *NPR1* and *PR1*, are central components of the salicylic acid (SA)-mediated systemic acquired resistance (SAR) pathway, while both *LOX1* and *PR3* are associated with the jasmonic acid (JA) signaling pathway (Vlot *et al.,* 2009; Thaler *et al.,* 2012**).** Our findings indicate that the mechanisms in which the two employed vermicompost materials

enhance resistance in plants involve triggering different defence signaling pathways with different levels yet interconnected.

On the other hand, data showed a slight up-regulation in PAL expression in plants treated with vermicompost. The relative up-regulation was significant in plants treated with FVC and challenged with the pathogen while plants treated with CVC and the pathogen didn't show a significant up-regulation in PAL expression. Phenylalanine ammonia-lyase (PAL) plays crucial role in plant defence against pathogens as it is involved in the production of various compounds such as lignin, flavonoids, and phytoalexins (MacDonald and D'Cunha, 2007; Caretto *et al.,* 2015).

CONCLUSION

The application of cow dung and dish slug vermicomposts proved effective in suppressing damping off in cucumber plants. and significantly promoted their growth as evidenced by increased shoot and root dry weights. This efficacy was linked to the involvement of various biochemical and molecular mechanisms. These findings highlight the value of vermicomposts, particularly CVC, in enhancing plant productivity and health.

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فعالية الفيرميكمبوست في مكافحة مرض موت البادرات الناجم عن فطر الرايزوكتونيا في الخيار

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تم تحضير نوعين من الفيرميكمبوست من روث الأبقار ومخلفات الأسماك وذلك باستخدام خليط من ثلاثة أنواع من ديدان الأرض وهي:*Perionyx excavates , Lumbricus rubellus, Eisenia fetida* . تم تقييم كفاءة الفيرميكمبوست المنتج في مقاومة مرض موت البادرات أسفل التربة وفوق سطح التربة في نباتات الخيار والمتسبب عن فطر*Rhizoctonia solani*. أجريت تجارب في الأصص على مدار عامين (٢٠٢٢-٢٠٢٣ و٢٠٢٣-٢٠٢٤). وجد ان كلاً من فيرميكمبوست روث الابقار ومخلفات الأسماك قد أدى الى خفض الإصابة بالمرض بدرجة معنوية وأيضاً الى تحفيز نمو النباتات في صورة زيادة للوزن الجاف للجذور والمجموع الخضري. وكان فيرميكمبوست روث الابقار هو الأقوى في تحفيز انتاج المركبات الفينولية مقارنة بباقي المعاملات. وقد تفوق فيرميكمبوست روث الابقار على فيرميكمبوست مخلفات الأسماك في تثبيط مستويات DPPH لكن كلاهما أظهر تأثيرات معنوية. بالإضافة الى ذلك فان كلا النوعين من الفيرميكمبوست قد أدى الى زيادة في نشاط انزيمات البيروكسيديز والكتاليز مقارنة بالنباتات الغير معامله. وأوضحت النتائج حدوث زيادة في مستوى تعبير جينات المرضية (NPR 1SA , PR1) و (LOX1 JA , PR3)و Phenylalanine ammonia-lyase (PAL) وذلك في النباتات في معظم المعاملات المرتبطة بالنوعين المستخدمين من الفيرميكمبوست. ووجد أن مستوى التعبير الجيني ل NPR1 قد أرتفع بشكل معنوي في النباتات المعاملة بفيرميكمبوست روث الابقار والتي تم معاملتها مسبقا بالفطر حدثت زيادة معنوية في مستوى التعبير للجين PR1 النباتات المعامله بكلا من فيرميكمبوست روث الابقار و فيرميكمبوست مخلفات الأسماك والتي تم معاملتها مسبقاً بالفطر *R. solani*. وكان فيرميكمبوست مخلفات الأسماك هو الأكثر كفاءة في تحفيز التعبير الجيني لـ LOX1 في حين كان تأثير كلا من فيرميكمبوست روث الابقار ومخلفات الأسماك متقارب معنوياً في تحفيز التعبير الجيني لـ3PR . لم يكن تأثير فيرميكمبوست مخلفات الأسماك معنوي على تعبير الجين PAL في حين أدت المعاملة بفيرميكمبوست روث الابقار الى انخفاض في التعبير الجيني مقارنة بالنباتات الغير معاملة.

الكلمات المفتاحية: الخيار، فيرميكومبوست، موت البادرات ، ريزوكتونيا، نشاط إنزيمات الدفاع، الجينات المرتبطة بالدفاع.