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GREEN ALTERNATIVES: EXPLORING THE NEMATICIDAL AND ANTIOXIDANT POTENTIAL OF GARLIC STALK EXTRACTS

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ABSTRACT

The widespread use of synthetic chemicals in agriculture has led to significant environmental hazards. In response to this researchers are exploring plant-based materials as a more eco-friendly approach to agricultural practices. This study was aimed to investigate the nematicidal potential of garlic stalk extracts (aqueous, ethanolic, and fermented) against the root-knot nematode *(Meloidogyne incognita)* and evaluate their phenolic, flavonoid, and antioxidant contents. The results indicated that increasing concentration of the extracts led to higher immobility and mortality rates of *M. incognita* juveniles. The ethanolic extract was the most effective achieving 100% juvenile mortality, at concentrations of 50 and 75% after 72 hours. The fermented extract also showed promising result with mortality rates increasing from 72% to 94% after 72 hours. In contrast, the aqueous extract of garlic stalk exhibited significantly lower toxicity, with mortality rates ranging from 64% to 86% against *M. incognita.*

Phytochemical analysis revealed that both ethanolic and aqueous extracts exhibited significant amounts of total phenolics and total flavonoids, demonstrating high antioxidant activity. Conversely, the fermented extract showed the lowest levels of total phenolics, total flavonoids, and exhibited the lowest antioxidant activity.

Key Words: Garlic stalks, Aqueous extract, Ethanolic extract, Fermented extract, Nematicidal activity, *Meloidogyne incognita.*

INTRODUCTION

The detrimental environmental impact of synthetic chemicals in agriculture has spurred a growing interest in exploring plant-based alternatives. Driven by the need to minimize environmental harm and promote sustainable agricultural practices, researchers are actively

investigating the potential of plant-derived materials to provide safer and more environmentally benign solutions. Garlic (*Allium sativum* L.), a plant with a long history of medicinal use, is particularly rich in organosulphur compounds. These compounds are believed to contribute significantly to its health-promoting properties **(Shang *et al*., 2019**)

Traditionally, garlic and its related compounds have been reported to have several biological activities including anticarcinogenic, antioxidant **(Rahman and Lowe 2006)**, antidiabetic, renoprotective, anti-atherosclerotic, antibacterial, antifungal **(Davis, 2005)** and antihypertensive activities **(Badal *et al.* 2019)**. Moreover, garlic has been used in traditional medicine to treat indigestion, respiratory and urinary tract infections and cardiac disorders. It has shown carminative, antipyretic, sedative, aphrodisiac, and diuretic effects **(Souza *et al*., 2011).** Chemical fumigation , particularly with methyl bromide (MeBr), has been a common practice for many years. However, the Montreal Protocol has banned the production and use of MeBr globally in 2015 **(Ajwa *et al*., 2003).** This ban will have a significant impact on agricultural production unless safe and effective alternatives are developed. Furthermore, human health concerns are fueling steady growth in demand for organic produce in many countries. With conventional pesticides off-limits, safe and ecologically sound control strategies are a necessity (**Tabarant *et al.*, 2011** and **Radwan *et al*., 2012)**. In particular, decomposition of certain plant residues releases active compounds that kill pests without lingering on produce, as is the case with some synthetic compounds. Among the most widely studied, several liliaceous crops, such as *Allium sativum* L., *A. cepa* L. and *A. fistulosum* L., contain sulphur compounds that are hydrolyzed to form a variety of isothiocyanates with broad insecticidal, nematicidal, fungicidal, antibiotic and phytotoxic effects **(Choi *et al*., 2007).** A wealth of literature has been compiled expressing that *A. sativum* residues can reduce the damage to vegetable crops by nematode and other soilborne diseases (**Park *et al*., 2005 and Choi *et al*., 2007**).

Plant parasitic nematodes can cause damage to plants that is often subtle and may be confused with nutritional deficiencies (**Trambadiya *et al*., 2023**). Out of the hundreds of different nematode species that can infect plants, only a few are economically significant as root-feeding diseases. If there are a large number of damaging nematodes present, plant growth will suffer (**Desai, 2007 and Jones *et al*., 2013**). Many plants are affected by the root-knot nematode *Meloidogyne* incognita (Kofoid and White) Chitwood. It is extremely harmful to crops, causing significant damage and decreased productivity. Nematodes negatively impact the host plant's normal physiology, growth, and development during parasitism. This phenomenon has been linked to the host's direct and indirect response to the nematode's mechanical and biochemical activities (**Bhargava *et al*., 2007**).

Most research has focused on garlic bulbs, while garlic stalks have received negligible attention. This study was aimed to investigate the potential of garlic stalks as a green alternative for managing root-knot nematode *(M. incognita*) and to explore their value as a source of phenolics, flavonoids, and antioxidant properties.

MATERIALS AND METHODS

Preparation of plant material

Fresh garlic stalks were collected, washed, and dried . The stalks were divided into three equal parts, each weighing approximately 200 g. One part was used for aqueous extraction, another for ethanolic extraction, and the third was fermented using specific bacteria.

Preparation of aqueous extract

Aqueous extraction of garlic stalk was carried out using a modified method based on **Muhamad and Mat, (2019)** and **Sirisa-ard, *et al*., (2023)**. Briefly, 200 grams of freshly minced garlic stalks were subjected to two successive extractions with 1000 mL of distilled water. The first extraction involved boiling for 20 minutes followed by 24 hours of incubation at room temperature. The second extraction was left to incubate at room temperature for 24 hours After each extraction, the mixture was filtered and the combined filtrates were dried in an oven at 40°C for 72 hours. The resulting dried extract film was weighed and reconstituted in distilled water to achieve a final concentration of 35 mg/ml.

Preparation of ethanolic extract

A modified protocol based on **Oroian *et al.* (2020),** was used to extract compounds from 200 g of freshly minced garlic stalks. Approximately 1000 mL of 70% ethanol was added to 200 g the minced garlic stalks and sonicated in an ultrasonic water bath for 30 minutes. The mixture was filtered and the remaining plant material was underwent a second 30-minute sonication with an additional 1000 mL of 70% ethanol to enhance extraction. The combined filtrates were then dried in an oven at 40°C for 72 hours resulting in a dried film that was reconstituted in distilled water to achieve a final concentration of 35 mg/ml extract.

Preparation of fermented extract

Extracellular enzyme containing supernatants from *Pseudomonas mendocina* were prepared as described above.An enzymatic solution (500 mL) was added to 200 g of minced garlic stalks, then mixed and adjusted to pH 7.0. The solution was shaken in a reciprocating shaker for 48 h at 37 °C. Subsequently, the solution was filtered through Whatman filter paper No 1 and dried in an oven at 40°C for 72 hours. The obtained dried film was reconstituted in distilled water to a final volume of 200 mL.

Determination of total phenolics content

Total phenolic content was determined using a modified Folin-Ciocalteu method adapted from **Mohammed and Manan (2015).** Briefly, 100 µL of the

extract was mixed with 300 µL of Folin-Ciocalteu reagent and 3 mL of distilled water. After 10 minutes incubation at room temperature, 1 mL of 20% (w/v) sodium carbonate solution was added to the mixture. The mixture was then incubated in the dark for 2 hours. The absorbance of the resulting-colored complex was measured at 765 nm using a spectrophotometer. A blank sample containing distilled water instead of the extract was used as a control. A standard curve was prepared using known concentrations of gallic acid (0-200 mg/L). Total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per ml of extract.

Determination of total flavonoids content

Total flavonoid content was determined using a modified method based on **Kumar *et al.* (2015**). Briefly, 0.5 mL of the extract was mixed with 1.5 ml of 10% aluminum chloride, 100 µL of 1 M potassium acetate, and 1mL of deionized water. The mixture was incubated at room temperature for 30 minutes. The absorbance of the resulting complex was measured at 415 nm using a spectrophotometer. A standard curve was prepared using known concentrations of quercetin (ranging from 0 to 50 mg/L). Total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per ml of extract.

Determination of antioxidant activity

The DPPH scavenging assay was performed with modifications based on the method described by **Baliyan (2022) .**Briefly, 0.5 mL of the stalk extract was mixed with 0.5 mL of DPPH solution (60 µM in absolute ethanol). The mixture was incubated in the dark for 40 minutes. The absorbance of the resulting mixture was then measured at 517 nm using a spectrophotometer

The percentage of antioxidant activity was calculated using the following formula:

$$\% \text{ of antioxidant activity} = [(Ab - As) \div Ab] \times 100$$

where: Ab, is the absorbance of the blank and As, is the absorbance of the sample, **(Baliyan, 2022).**

Bacterial strains and growth conditions

The enzymatic solution was obtained from a bacterial strain previously isolated and identified as *P. mendocina* (provided by the microbiology lab., at Desert Research Center). The cellulolytic bacteria were prepared in a nutrient broth medium (0.5% yeast extract, 0.5 % peptone, and 1 % glucose) at 30°C for 24 h. The cellulolytic microorganism isolates were used to inoculate 100 mL of liquid medium CMC-Na (Mw, 1200) containing 10 g, of peptone, 0.5 g of $MgSO_4$, 1 g of KH_2PO_4, 1 g of Na_2HPO_4 and 1000 mL water pH 7.0 at 37 °C. After 30 hours of culture, the broth was separated by centrifugation (8,000 g, 10 min) at 4 °C and the supernatant was collected.

Culture preparation of *M.incognita*

A pure stock culture of the root-knot nematode *M. incognita* (Kofoid and White) Chitwood was prepared and propagated under greenhouse

conditions to provide a source of second-stage juveniles (J2s). A single egg mass isolated from infected eggplant was used to inoculate young eggplant seedlings of the susceptible cultivar [eggplants (Solanum melongena)] to establish a nematode population. This technique was repeated to obtain enough egg masses. Species identification was determined by examining the perineal pattern system of adult females as described by **Eisenback *et al.*, (1981**). Second stage juveniles were obtained by incubating egg masses in Petri- dishes containing sterile distilled water. Newly hatched juveniles were collected using a micropipette.

Determination of Nematicidal Activity

Effect of garlic *(Allium sativum)* stalk extracts on juvenile (J2) mortality of *M. incognita* was studied . The mortality test was conducted under in-vitro conditions. One ml of nematode suspension containing 50 freshly hatched juveniles of *Meloidogyne incognita* was added to constant volume of garlic *(Allium sativum* extracts (Aqueous, ethanolic and fermented) for the test, desired concentrations (25, 50 and 75 of leaf extracts into petri dishes (80mm) and 50 freshly hatched second stage larvae of *M. incognita* in 5 ml distilled as control. All dishes were incubated in an incubator at (25±2°C). After 24, 48 and 72 h the juveniles were counted for mortality and non- mortality under stereoscope microscope. The death of nematodes was confirmed by keeping them in tap water for 24 h. The per cent mortality was worked out from an average of three replicate. The percentage of juvenile mortality was calculated using Abbott's formula **(Abbott, 1925)** as follows: Mortality (%) = [(mortality percentage in treatment – mortality percentage in control) /(100 –mortality percentage in control)] × 100

Statistical Analysis

Statistical analysis was conducted based on triplicate measurements using a one-way ANOVA and subsequent Duncan's multiple range test in the SPSS 17.0 program. Statistically significant differences ($P \leq 0.05$) were identified between the control and experimental groups.

RESULTS

Based on the data in Table (1), garlic (*A. sativum*) stalk extracts exhibited varying levels of nematicidal activity against *M. incognita* juveniles .All extract concentrations resulted in nematode mortality compared to the control. Higher concentrations and longer exposure times led to increased mortality of *M. incognita* juveniles. The highest mortality rate of M. incognita J2s (100%) was observed at a 75% ethanolic extraction concentration of garlic extracts after 48 and 72 hours, followed by 50% at 72 hours. The bacterial extract showed 94% mortality after 72 hours, while the bacterial extract at 50 % concentration recorded 84 % mortality. After 24 hours, the bacterial extract and aqueous solution exhibited minimum mortality rates of 30 and 50% at 25 %concentrations, respectively, while the bacterial extract at 50% concentration recorded 52% mortality.

Table (1). Effect of garlic *(Allium sativum*) stalk extracts on mortality of *M. incognita* juveniles (J2s)

Extract	Concentration %	Time					
		24h		48h		72h	
		No. of immobile juvenile	Mortality (%)	No. of immobile juvenile	Mortality (%)	No. of immobile juvenile	Mortality (%)
Aqueous	25	15.00 ± 1.55^{a}	30.00	23.00 ± 1.73^{a}	46.00	32.00 ± 1.14^{a}	64.00
	50	25.00 ± 1.10^{c}	50.00	33.00 ± 0.67^{b}	66.00	35.00 ± 0.97^{b}	64.00
	75	28.00 ± 1.45^{c}	56.00	37 ± 1.45^{b}	74.00	43.00 ± 0.44^{c}	86.00
Fermented	25	18.00 ± 0.88^{b}	36.00	25.00 ± 1.45^{a}	50.00	36.00 ± 1.15^{ab}	72.00
	50	26.00 ± 1.66^{c}	52.00	3400 ± 1.00^{b}	68.00	42.00 ± 1.35^{b}	84.00
	75	35.00 ± 1.17^{d}	70.00	39.00 ± 1.45^{c}	78.00	47 ± 2.03^{cd}	94.00
Ethanolic	25	33.00 ± 1.83^{d}	66,00	39.00 ± 1.18^{c}	78.00	47.00 ± 1.15^{d}	90.00
	50	37.00 ± 0.59^{e}	74.00	48.00 ± 1.18^{d}	96.00	50.00 ± 1.55^{f}	100.00
	75	48.00 ± 0.45^{ef}	96.00	50.00 ± 0.00^{f}	100.00	50.00 ± 0.00^{f}	100.00
Control		00	00	000	00	00	00
F-value		77.566		55.950		33.198	
P-value		<0.001		<0.001		<0.001	

The values represent mean ± SE. Different letters within the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test.

Data in Figure (1) compare the antioxidant activity, total phenolics, and total flavonoids of three types of garlic extracts aqueous, ethanolic, and fermented. The aqueous extract exhibited the highest antioxidant activity at (95.30%), followed by ethanolic extract at (89.27%), and fermented extract at (60.10 %). The aqueous extract (9.42 mg QE//ml) and ethanolic extract (9.35 mg QE//ml) had significantly higher total phenolics content than the fermented extract (3.82 mg QE//ml). The ethanolic extract showed the highest total flavonoid content at 3.54 mg GE//ml, while the fermented extract had the lowest at 1.00 mg GE/ml. The aqueous extract had a moderate total flavonoid content of 2.63 mg GE/ml, falling between the ethanolic and fermented extracts.

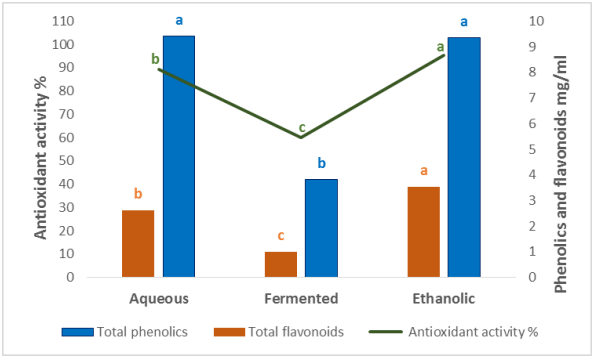

Fig (1). Total phenolics,total flavonoids and antioxidant activity of garlic stalk extracts. Different letters represent significant differences ($P \leq 0.05$) according to Duncan's multiple range test.

DISCUSSION

Garlic is a popular vegetable crop known for its potent antimicrobial, anticoagulant, antithrombotic, and anticancer properties leading to increase in production in recent years. The large amounts of garlic straw produced can serve as a valuable source of compounds that inhibit harmful soil microorganisms. In a study by **Morsy *et al.* (2009)** promising results were found against *Fusarium solani.*

Eder *et al* (2021), reported a strong nematicidal activity on *M. incognita* second stage juveniles (J2s) using of a granular garlic extract formulation. This formulation contained the same technical grade material as the liquid formulation tested in our experiments. In earlier studies, **Sukul *et al*.(1974**) reported a 61% mortality of *M. incognita* J2s, even after a 5 min exposure to a garlic ethanol extract, as well as a garlic aqueous extract which killed 100% of *M. incognita* J2s within 72 h (**Gupta *et al*. (1985**) and **Tesfaye and Mengesha (2015).** Additional studies described a strong reduction of *M. incognita* egg hatching and J2s mortality by treatments with allicin, i.e., the precursor of polysulfides contained in the a commercial garlic extract formulate (GEF) The Global Environment Facility tested in this study (**Gupta and Sharma 1993**).

The mode of action of diallyl polysulfides, the main active compounds in garlic-derived nematicides, is still unclear, but their multi-site activity is likely. Studies by **Sparks *et al*. (2020**) and **Chatterji *et al*., (2005**) have shown DNA damage and cell apoptosis due to reactive oxygen species formed by polysulfide compounds. **Anwar *et al.* (2014)** suggested that the metal-binding capacity of diallyl polysulfides could disrupt metal homeostasis**. Gupta and Sharma (1991**), found that an aqueous extract of garlic bulbs suppressed the hatching of

Meloidogyne incognita eggs. Plant products are increasingly being studied as prophylactics against plant-parasitic nematodes.

The antioxidant activity of garlic extract was assessed using the DPPH radical scavenging assay, a widely employed method due to its reliability and ease of use (**Bhandari *et al.*, 2014**).The data suggests that all three extracts (aqueous, ethanolic, and fermented) possess significant antioxidant activity, with the aqueous extract demonstrating the highest activity at 95.30% followed by the ethanolic extract at 89.27%. These findings indicate that these extracts are highly effective at scavenging free radicals, which is crucial for preventing oxidative damage and promoting overall health (**Chen *et al*., 2013** and **Guevar**a ***et al.*, 2019).**

Phenolic compounds are known for their antioxidant properties and contribute to the health benefits of various foods. They play a role in anti-inflammatory, anti-cancer, and cardioprotective effects (**Rocchetti *et al*., 2022**). Both the aqueous and ethanolic extracts had significantly higher total phenolic contents compared to the fermented extract, suggesting that the aqueous and ethanolic extracts are richer sources of beneficial phenolic compounds, which may correlate with their higher antioxidant activity. Previous studies have shown that the total phenolic content varies from 5.63 mg GAE/g in aged garlic extract to15.23 mg GAE/g FW **(Chen *et al*.,2013).**

Flavonoids, a class of phenolic compounds, are well-known plant antioxidants with diverse biological activities including anti-inflammatory, anti-allergic, and radical-scavenging properties, as well as contributions to cardiovascular health (**Ullah *et al*., 2020 and Rakha *et al*., 2022).** The ethanolic extract had the highest total flavonoid content, indicating potential health benefits associated with flavonoids. In contrast, the fermented extract had the lowest content , suggesting that fermentation process may have led to the degradation or transformation of some of the bioactive compounds, resulting in a decrease in antioxidant potential.

CONCLUSION

The data demonstrates that all garlic stalk extracts exhibited nematicidal activity against root-knot nematodes (*Meloidogyne incognita*), with the ethanolic and fermented extracts displaying the most potent effects compared to the aqueous extract. The aqueous and ethanolic extracts also demonstrated strong antioxidant properties, due to their high total phenolics and flavonoids content. While the fermented extract showed lower antioxidant activity, it still contained bioactive compounds. Further research is necessary to fully understand the phytochemical composition of each extract and explore their potential applications.

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البدائل الخضراء: استكشاف الامكانيات السامة للنيماتودا ومضادات الأكسدة لمستخلصات سيقان الثوم

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أدى الاستخدام الواسع النطاق للمواد الكيميائية المصنعة في قطاع الزراعة إلى مخاطر بيئية كبيرة، وإدراكًا للحاجة الملحة إلى بدائل مستدامة. لذلك يسعي الباحثون للبحث عن مواد نباتية كنهج أكثر صداقة للبيئة في الممارسات الزراعية.

هدفت هذه الدراسة إلى التحقق من قدرة مستخلصات ساق الثوم (المائي والإيثانولي والمخمر) ضد نيماتودا تعقد الجذور واستكشاف قيمتها كمصدر للفينولات والفلافونويدات ونشاط مضادات الأكسدة.

أشارت النتائج إلى أن عدم الحركة ونسبة الموت ليرقات نيماتودا تعقد الجذور ***Meloidogyne incognita*** زادت بشكل ملحوظ مع زيادة تركيزات المستخلص، وكان المستخلص الإيثانولي لساق الثوم أكثر فعالية في موت اليرقات، حيث وصل معدل الموت إلى 100% عند التركيزات 50 و75% بعد 72 ساعة. بعد ذلك، أدى المستخلص المخمر لساق الثوم إلى زيادة معدلات الموت لليرقات من 72% إلى 94% بعد 72 ساعة، وفي المقابل أظهر المستخلص المائي لساق الثوم سمية أقل بكثير، حيث تراوحت معدلات الموت من 64% إلى 86% .

أظهر التحليل الكيميائي النباتي أن كلا من المستخلصات الإيثانولية والمائية تحتوى على كميات كبيرة من الفينولات الكلية والفلافونويدات الكلية وبالتالي إظهار نشاط مضاد للأكسدة عالي، وعلى العكس من ذلك أظهر المستخلص المخمر أدنى مستويات من الفينولات الكلية والفلافونويدات الكلية وأدنى نشاط مضاد للأكسدة.