



RESEARCH ARTICLE

Effect of selected plant extracts on the viability and vigour of maize seeds infected with *Fusarium semitectum*

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Abstract

This study aimed to investigate the role of various plant extracts: neem leaf extract (*Azadirachta indica* A. Juss.) and cold and hot aqueous fruit extracts of the chinaberry tree (*Melia azedarach* L.), referred to as A1, A2, A3 respectively, besides the control treatment A0 at concentrations of 0, 500, 1000, 1500, 2000, 2500 mg L⁻¹, for controlling the fungus *F. semitectum*. Based on preliminary screening, effective concentrations were identified, namely 1000, 2000 and 1500 mg L⁻¹ for A1, A2 and A3 respectively, which resulted in 100 % inhibition of fungal growth. These were then used for conducting a field test experiment and laboratory test to study the effect of soaking seeds using various combinations of A0, A1, A2, A3. Four experiments were conducted on the germination and emergence of maize seeds in a media with fungus culture. Results showed that neem leaf extract at a concentration of 1000 mg L⁻¹ and the aqueous extracts of chinaberry fruits at a concentration of 1500–2500 mg L⁻¹ completely inhibited the growth of the fungus. Moreover, neem treatment alone A1 showed better results over the control treatment in both *in vitro* germination (87.50 %) and emergence (80.25 %). Interaction A1×A2 showed maximum strength of germination (1135), emergence length (6.72 cm), maximum dry weight of sprouting (0.0517g), suggestive of a synergistic effect of the compounds in A1 and A2. These observations reiterate that neem and safflower leaf extracts could be utilized effectively in promoting healthy seed life with reduced possibilities of seed diseases, besides being eco-friendly substitutes for poisonous pesticides.

Keywords: *Fusarium semitectum*; plant extracts; seed viability; sustainability; *Zea mays*

Introduction

Maize (*Zea mays* L.) is one of the world's most important strategic crops due to its high nutritional and economic value. It is used in human and animal feed and in the production of oils, starch and ethanol. However, its productivity suffers sharp declines due to seed and seedling infection by several pathogenic fungi, most notably those belonging to the genus *Fusarium*, particularly *Fusarium semitectum* is one of the most important diseases causing seed rot and seedling death before or after germination in many countries worldwide (1).

Fungi live in various environments alongside many bacteria and other organisms. To resist and survive, they possess several important strategies, such as producing metabolites against these organisms (2). These metabolites are often transferred from the soil to the plant and cause yield reduction (3). Fungi belonging to the genus *Fusarium* are among the most important pathogens affecting maize at different growth stages, causing seed and root rot and seedling death (4). Specifically, the fungi of the species *F. semitectum* and *Fusarium* species and *Verticillium* spp. are among the most widespread fungi in warm, humid fields (5). This fungus causes significant losses in seed quantity and quality due to reduced

viability and germination. It also contaminates seeds with mycotoxins, such as fumonisins, which pose a risk to humans and animals (6). Disease losses estimated in the United States indicate that during 2019, *Fusarium* spp. diseases resulted in a reduction of more than 146 million bushels of maize production due to stalk rot, while losses due to ear rot reached about 91.7 million bushels, reflecting the important economic impact produced by diseases of this fungal genus on maize crop productivity (7).

While most farmers rely on chemical pesticides to control fungal infections, environmental and health problems have arisen from the accumulation of these substances in the agricultural environment, water and soil, in addition to the development of pesticide-resistant fungal strains (8). Consequently, recent research has focused on finding safe and effective natural alternatives to chemical pesticides, including the use of plant extracts containing organic compounds with antifungal activity, such as azadirachtin in neem and terpene and phenolic compounds in sabkha (a type of *Sorghum bicolor* L.).

These studies indicate that such extracts might be inhibiting the growth of pathogenic fungi and improving germination and emergence characteristics upon treatment of seeds. Hence a

sustainable option which is in line with the goals of sustainable agriculture. Several uses were reported for neem (*Azadirachta indica* A. Juss.) leaf extracts, which earned the plant the title "miracle tree." Neem seeds represent natural insecticides, fungicides and antibacterial agents because they contain azadirachtin, salanin and nimbin (9). Additionally, fruit extracts from the chinaberry tree (*Melia azedarach* L.) are effective as antibacterial, antifungal and even some types of viruses, including the coronavirus, due to their phenolic and flavonoid content (10).

These extracts are naturally characterised and rapidly biodegradable materials, making them environmentally and economically safer as compared to chemical pesticides. In addition, recent studies have demonstrated that the efficacy of the extracts is influenced by the preparation method, either cold or hot, the concentration of the active ingredient and the nature of the extracted compounds (11). In light of the aforementioned, the present study aimed at effectively testing the application of local plant extracts-neem leaves and cold and hot senna fruits-for chemical pesticides in the control of the fungus *F. semitectum* with a view to contributing to the support of biological control programs and the move towards sustainable agriculture and investigating their effect on the viability of maize seeds and seedling activity.

Materials and Methods

To achieve the goals of sustainable agriculture and protect the environment from chemical pollutants, three experiments were conducted 2 laboratories and 1 field-to determine the effect of certain plant extracts and their different combinations in controlling the fungus (*Fusarium semitectum*) associated with maize seeds (Buhoth106 cultivar). The first two laboratory experiments aimed to determine the effect of different concentrations of plant extracts (neem leaf extract and cold and hot aqueous extracts of (*Salvia officinalis*), designated A1, A2 and A3, respectively, in addition to a control treatment (A0) at concentrations of 0, 500, 1000, 1500, 2000 and 2500 mg L⁻¹, on controlling the fungus *Fusarium semitectum*. The lowest concentration of these extracts, which gave an effective inhibitory effect on the fungus, was selected. For the three extracts, the concentrations were 1000, 2000 and 1500 mg L⁻¹ respectively. These concentrations resulted in the highest inhibition for each extract. The highest inhibition rate of 100% and those extracts at the selected concentrations were introduced into a field experiment (field germination experiment) and another laboratory experiment (second laboratory experiment) to find out the effect of soaking the seeds with those extracts in their different combinations (Table 1) on germination and seedling vigour of maize seeds in a medium contaminated with the fungus (*F. semitectum*).

Table 1. Study coefficients and their symbols

Treatment	Symbols
Distilled water (control treatment)	A0
Neem leaf extract	A1
Cold aqueous extract of <i>Rosa canina</i>	A2
Hot aqueous extract of <i>Rosa canina</i>	A3
Neem leaf extract × cold aqueous extract of <i>Rosa canina</i>	A1×A2
Neem extract × hot aqueous extract of <i>Rosa canina</i>	A1×A3
Cold aqueous extract of <i>Rosa canina</i> × hot aqueous extract of <i>Rosa canina</i>	A2×A3
Neem leaf extract × cold aqueous extract of <i>Rosa canina</i> × hot aqueous extract of <i>Rosa canina</i>	A1×A2×A3

Isolation and Identification of the fungus *F. semitectum*

Seeds of this variety were obtained from the autumn harvest of 2023, grown in the fields of the Yellow Maize Research Station of the Iraqi Ministry of Agriculture. Samples were taken from ears showing symptoms of fungal infection and isolated and identified at the Pathology Laboratory of the Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. The harvested seeds were manually deseeded and 400 seeds were taken from each sample for fungal isolation. The grains were surface sterilised with a 1% sodium hypochlorite solution for 2 min, washed with sterile distilled water and then dried with sterile filter paper. The seeds were sown, five seeds per sample, in sterile 9 cm diameter glass Petri dishes containing 15–20 cm³ of potato sucrose agar (PSA) culture medium (200 g potato + 10 g sucrose + 20 g PSA + 1 L of distilled water). The plates were incubated at 25 ± 1 °C for 7 days (12). All seeds were then examined under the minimum power of the compound microscope and the different fungi were purified and identified to the species level based on culture and morphological characteristics and following the approved taxonomic keys (13). As for the species of the genus *Fusarium*, they were purified by the single spore method inside Petri dishes according to the modified method reported earlier (14) and incubated on a PSA medium for 24 hr at a temperature of 25 ± 1 °C. The dishes are examined after 24 hr and the colonies are identified with a needle. Each developing spores are then transferred to a dish containing the PSA culture medium and 4 duplicate samples are used. After its growth is complete, it selects the colony that represents the mushroom farm, neglects other dishes and this colony is considered the foundation and kept in the soil and re-isolated according to the requirements of the research. The species was identified after cultivation on carnation leaf-piece agar (CLA), potassium chloride agar (KCl agar) and PSA, following the established taxonomic keys (15).

Collection and preparation of plant extracts

The fruits of the trees, *M. azedarach* were collected from the gardens of the College of Agricultural Engineering Sciences, University of Baghdad (Abu Ghraib area) at the end of April 2023. The leaves of the neem tree (*A. indica*) were also collected from trees planted on the College site in Jadriya. The fruits and leaves were cleaned of dirt and impurities and dried in the shade at room temperature, turning them periodically to prevent fungal growth. After drying, the samples were ground in a Wiley Mill electric grinder and the powder was sieved through a 50–60 mm sieve. The powder was stored in sealed plastic bags at 4 °C until use. The following method was adapted from previous studies (16), prepared cold and hot aqueous extracts of neem fruit and an aqueous extract of neem leaves.

Cold aqueous extract of neem leaves (A1) and chinaberry fruit (A2)

Fifty gram of powdered neem leaves and chinaberry fruit were weighed and 500 mL of distilled water was added to a 1 L glass flask. The mixture was shaken with a magnetic stirrer for 15 min and then left to stand for 24 hr at room temperature. The resulting solution was filtered through double gauze and then filter paper and the filtrate was centrifuged at 3000 rpm for 10 min to obtain a clear solution. The filtrate was dried at laboratory temperature to obtain the dry matter and then stored in the freezer until use. The average dry matter yield was 0.7962 g per 50 g of powder.

Hot aqueous extract of chinaberry fruit (A3): was prepared using the same method, except that hot water (80–90 °C) was used.

This experiment was conducted to assess the effectiveness of plant extracts in inhibiting fungal growth on nutrient agar. The experiment was carried out according to a completely randomized block design (CRBD) with 4 replicates. Calculated amounts of each extract were added to PSA before solidification to obtain final concentrations of 500, 1000, 1500, 2000 and 2500 mg L⁻¹, calculated on a dry matter basis. The medium was poured into 9 cm diameter Petri dishes at a volume of 20 mL per dish. After solidification, the dishes were inoculated with a 0.5 cm diameter disc taken from the edge of a 5-day-old *F. semitectum* colony. The control treatment (A0) contained the medium. Potato sucrose agar only, without extract. The plates were incubated at 25 ± 2 °C for 5 days and the diameter of the fungal colony was measured on 2 perpendicular axes.

The inhibition percentage was calculated using the equation:

$$\text{Inhibition percentage} = [(\text{Colony diameter in control} - \text{Colony diameter in treatment}) / \text{Colony diameter in control}] \times 100$$

Testing the effect of plant extracts on the viability and germination of maize seeds in fungal-contaminated medium

After determining the most effective concentrations for *in vitro* inhibition, the selected concentrations (1000 mg L⁻¹ for neem, 2000 mg L⁻¹ for cold saffron and 1500 mg L⁻¹ for hot saffron) were used in two experiments:

Second laboratory experiment

Testing seed viability

Intact seeds were sterilized with a 1 % sodium hypochlorite solution for two min, then washed with distilled water and dried. The seeds were then soaked in solutions of the various extracts and combinations for 2 hr and finally distributed into germination dishes containing artificially contaminated soil with the fungus (*F. semitectum*) at a concentration of 1 × 10⁶ spores mL⁻¹.

The plates were incubated at 25 ± 1 °C and standard *in vitro* germination parameters were recorded, including:

Field experiment: Field emergence test

Treated and untreated seeds were sown in washed and solarized soil in a randomized complete block design (RCBD) with 3 replications. The percentage of field emergence was recorded 10 days after sowing and seedling length and dry weight were measured.

Traits studied: The measured parameters included

- Final *in vitro* germination percentage (%): based on the number of normal seedlings 10 days after sowing (second count) (17), then converted to percentages.
- Radius and plumule length (cm): Ten normal seedlings were taken after the 10-day testing period. The radicle and plumule were then separated at their point of attachment to the seed and their lengths were measured using a ruler. The average lengths were calculated (17, 18).
- Seedling dry weight (mg): Seedlings with measured radicle and plumule lengths were placed in a perforated paper bag and dried at 80 °C for 24 hr. The average dry weight of each seedling was calculated (17, 18).

- Field emergence percentage (%): The percentage of emerging seedlings was measured 10 days after irrigation.

Statistical Analysis: Analysis of variance (ANOVA) was performed on the data using a one-factor RCBD for laboratory experiments, as the first laboratory experiment (effect of plant extracts on the size of the *F. semitectum* colony) involved the 3 extracts with 5 concentrations plus a comparison treatment. The second laboratory experiment (the effectiveness of plant extracts in the control of fungus infecting maize seeds). The field emergence experiment (applied with the same parameters as the second laboratory experiment) used a RCBD in its coefficients. The results were statistically analysed using Genstat software and then the results were compared using the LSD test at a significant level of 5 %.

Results

The results of the laboratory experiments showed that the 3 plant extracts (neem leaf extract, cold and hot aqueous extract of chinaberry) had a clear inhibitory effect on the growth of *F. semitectum*, the causative agent of damping-off disease in maize (Table 2). The degree of inhibition varies according to the type and concentration of the extract.

The results of the second experiment (testing seed viability in a fungal-contaminated medium) showed that treating seeds with plant extracts, either individually or in combination, led to a significant increase in germination and emergence characteristics compared to the control treatment (A0). The neem leaf extract treatment (A1) recorded the highest *in vitro* germination rate (87.50 %) and field emergence rate (80.25 %), while the lowest values were recorded in the control treatment (35 % and 20.25 %, respectively) (Table 3).

Table 2. Colony size of *Fusarium semitectum* fungus and percentage of inhibition by plant extracts

Plant extracts	Concentration (mg L ⁻¹)	Inhibition ratio (%)	Colony diameter (cm)
Neem extracts	0	0.00	9.00
	500	77.11	2.06
	1000	100.00	0.00
	1500	100.00	0.00
	2000	100.00	0.00
	2500	100.00	0.00
	LSD α 0.05	11.12	0.27
Fruits rosary trees extracts (cold aqueous)	0	0.00	9.00
	500	17.77	7.40
	1000	44.11	5.03
	1500	68.88	2.80
	2000	100.00	0.00
	2500	100.00	0.00
	LSD α 0.05	14.08	0.25
Fruits rosary trees extracts (cold aqueous)	0	0.00	9.00
	500	29.33	6.36
	1000	54.11	4.13
	1500	100.00	0.00
	2000	100.00	0.00
	2500	100.00	0.00
	LSD α 0.05	13.87	0.17

Table 3. Effect of plant extracts on the control of *Fusarium semitectum*, which infects maize seeds

Treatment	Field emergence (%)	Seed germination (%)	Radical length (cm)	Plumule length (cm)	Seedling dry weight (g)	Seedling power index
A0	20.25	35.00	7.33	6.00	0.0407	466
A1	80.25	87.50	6.12	6.21	0.0423	1082
A2	74.50	72.50	6.67	6.38	0.0469	946
A3	67.00	70.00	8.45	5.92	0.0384	1006
A1×A2	68.00	75.00	8.38	6.72	0.0517	1135
A1×A3	75.50	77.50	6.42	4.78	0.0487	866
A2×A3	77.00	80.0	8.80	7.00	0.0321	1264
A1×A2×A3	67.75	75.00	7.55	6.10	0.0534	1024
LSD α 0.05	5.14	7.66	NS	1.11	0.0032	272

Discussion

Effectiveness of plant extracts in inhibiting the growth of *Fusarium semitectum* under laboratory conditions

The neem leaf extract treatment achieved the highest inhibitory activity against fungal growth, reaching 77.11% at a concentration of 500 mg L⁻¹ and increasing to 100% at a concentration of 1000 mg L⁻¹ and above (Table 2). This effect has been attributed to the active principal compounds azadirachtin, salaniline and nimbin found in neem leaves, since these compounds are said to possess antifungal and anti-insectic properties. Such compounds prevent fungal mycelial growth through a mechanism of interference with protein and enzymatic syntheses that are involved in cell wall construction, thus completely inhibiting fungal colony development. Previous researchers confirmed that neem compounds have a broad-spectrum activity against several pathogenic fungi including *F. oxysporum*, *Rhizoctonia solani* and *Alternaria alternata* (19).

The results showed that the effectiveness of the cold aqueous extract was low at the concentration (500 mg L⁻¹) with an inhibition rate of 17.8%, but it gradually increased with the increase in concentration until the full inhibition rate (100%) reached at the concentrations of 2000 and 2500 mg L⁻¹. This behavior is attributed to the increased concentration of the water-soluble active compounds at higher levels, such as terpenes and phenolic compounds with fungal growth inhibitory effect. It has been known that during extraction, the limited solubility of some hydrophobic compounds, when conducted under low temperatures, may be responsible for this increase in concentration.

At lower concentrations, the hot aqueous extract exhibited better inhibitory activity than the cold extract, with an inhibition rate of 29.3% at 500 mg L⁻¹ and 54.1% at 1000 mg L⁻¹, reaching complete inhibition at 1500 mg L⁻¹ and above (Table 2). This is explained by the fact that heating favored the extraction of nonpolar, protein-bound compounds from fruit tissues, thereby increasing the concentration of active compounds that exert fungicidal activity. Another study reported that heating increases the efficiency of extraction of terpenoid and phenolic compounds, which have been clearly acting in the disruption of fungal cell membranes and causing ionic imbalance (20).

The general order of effectiveness of the extracts against fungi, from most effective to the least, was as follows: neem leaf extract > hot aqueous extract of chinaberry fruit > cold aqueous extract of chinaberry fruit. From this it follows that neem extract is more effective at lower concentrations and, therefore, a practical and economically feasible agent for fungal control in seed treatments compared with the other mentioned extracts. Results

correspond to data obtained by, where the authors have established that secondary compounds from the family Meliaceae to which belong neem and chinaberry exert a very high capacity to inhibit fungi and nematodes, at least partly through action mechanisms such as hyphae growth inhibition and protoplasmic coagulation of cells within these organisms (21).

Effect of plant extracts on the viability and germination of maize seeds

The observed effect may play a role in seed protection against pathogenic fungi, possibly by enhancing metabolic events responsible for the germination process, such as enzyme activity or breakdown of stored carbohydrates. This supports previous findings, that some plant extracts improve metabolic balance in seeds by suppressing surrounding microbial growth (22). The combination A1×A2 gave the maximum radicle and plumule length with means of 8.38 and 6.72 cm respectively when compared to other treatments, indicating an additive effect of active agents in the 2 extracts. Such stimulation in seedling growth can be attributed to a reduction in fungal stress, as well as increased activity of enzymes responsible for nutrient uptake from the seed, plus the hormonal role of certain plant compounds such as terpenes and flavonoids, which act as natural growth promoters.

The combination A1×A2 also gave the best results in dry weight of seedlings 0.0517 g and germination vigor index 1135 (Table 3), showing increased biomass, which is a result of good health and balance between root and plumule growth, in which also the combination was very good, thus giving its superiority in seedling dry weight. The high potency index shows the integrity of tissues and metabolism within the seed and the activity of the seedling and growth of its main parts, radicle and plumule. Thus, treatment of seeds with plant extracts demonstrates potential to be efficient in improving quality biological traits. Other treatments or combinations (A1×A3, A2×A3 and A1×A2×A3) showed partial improvements without significantly outperforming neem monotherapy in some traits (Table 3), confirming that neem remains the most effective component in both monotherapy and combinations. The current results are consistent with those of earlier studies, regarding the effectiveness of plant compounds in reducing seed-associated fungal damage (23). They also align with the observations of previous studies which demonstrated that extracts from plants (Meliaceae) possess dual biological activity: antifungal and growth-promoting (10, 21). This is because aromatic extracts from plants rich in essential oils have antifungal properties due to their content of phenolic compounds that interact with the proteins and membrane lipids of fungi.

Conclusion

The results of this study demonstrate that the use of neem extracts or their combinations, particularly neem leaf extract at a concentration of 1000 mg L⁻¹, is an effective and environmentally safe method for reducing the growth of the fungus *F. semitectum*, which causes seedling death in maize. It also contributes to improving germination, emergence and seedling vigour. Furthermore, the combination of leaves of neem and fruit of the chinaberry tree extracts results in a synergistic effect due to their content of different compounds (such as azadirachtin and phenols), which enhances antifungal activity and improves subsequent plant growth. The results recommend the adoption of these extracts as natural alternatives in integrated seed disease management programs within sustainable agricultural practices.

Authors' contributions

The experimental study was conducted by NMAH, SFMA, FAH and SHC. AFZA collected the data for conducting the research. SHC statistically analyzed the data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors have declared that there are no conflicts of interest.

Ethical issues: None

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