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Article *in* Notulae Botanicae Horti Agrobotanici Cluj-Napoca · March 2024

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Volume 52, Issue 1, Article number 13609
DOI:10.15835/nbha52113609
Research Article

In vitro multiplication, antimicrobial, and insecticidal activity of *Capparis spinosa* L.

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Abstract

Caper (*Capparis spinosa* L.) is a medical plant grown in Jordan. Mass harvesting of caper plants from their origin environments caused a reduction of these germplasm. Therefore, an easy and consistent method for clonal proliferation and callus induction was established for this species. *C. spinosa* L. *in vitro* culture affected in MS medium provided by 0.5 mg/L BAP gave 5.9 microshoots/explant. Two months later MS medium supplemented with 2.0 mg/L NAA developed a maximum callus induction of 33.1 mm. *Ex vitro, in vitro,* and callus growth of *C. spinosa* L. using ethanolic and methanolic extracts were tested for their antimicrobial activity against different species of bacteria and fungi. Both *ex-vitro* and *in vitro* plants exhibited similar antimicrobial activity. Maximum *ex vitro* plant antibacterial activity was (23 mm ± 0.58 inhibition zone) against *Staphylococcus epidermidi.* In comparison, callus extracts gave the highest antibacterial activity against *Bacillus cereus* and *Escherichia coli*. Moreover, caper plant extracts showed different antifungal effects against the tested fungi species. Investigation of the data showed that *ex-vitro* extract exhibited maximum antifungal activity compared to *in vitro* plants. Additionally, exposed *Bemisia tabaci* 4th nymphal instar to *C. spinosa* L. extracts suffered mortality ranging from 2 to 28%. In most instances, both ethanolic and methanolic extracts affected the survival of *B. tabaci* more than the control. The current study confirmed that *C. spinosa* L. has a wide range of antibacterial, antifungal, and insecticidal activity.

Keywords: antimicrobial activity; *Bemisia tabaci; Capparis spinosa* L.; *in vitro* multiplication; insecticidal activity; medical plant

Received: 06 Jan 2024. Received in revised form: 19 Feb 2024. Accepted: 14 Mar 2024. Published online: 22 Mar 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Jordan has a wide range of plant biodiversity due to its exceptional environment, combining Mediterranean and desert features. This valuable biodiversity faces threats from land conversion and habitat destruction, overgrazing and over-exploitation, increase in inhabitants, introduced species, pollution, overutilization of medicinal plants, and forest damage (Al Shhab *et al*., 2022; Shatnawi *et al.,* 2023). The significance of plants in managing infection is an ancient technique used for treating bacteria and fungi (Obeidat *et al*., 2011; Ibrahim, 2012; Shatnawi *et al*., 2021a). Thus, evaluation of local plants for their antimicrobial properties may yield valuable benefits (Shatnawi *et al*., 2022). *Capparis spinosa* L. is a vital remedial plant that is part of the Capparidaceae family. Caper usually flourishes in rocky and anhydrous habitats, fully exposed to the sun, and can withstand high temperatures (Shatnawi *et al*., 2011a; Moghaddas *et al*., 2012; Miransari and Smith, 2014; Kereša *et al*., 2019).

Propagating caper through seeds is not optimal, as seed germination rates are severely restricted, and seed coats contain inhibitors (Ramezani-Gask *et al*., 2008; Musallam *et al*., 2011; Al-Mahmood *et al.,* 2012; Miransari and Smith, 2014; Awatef *et al.,* 2017). The high collection of caper plants from their natural environments has led to a rapid reduction in their germplasm. Propagating this plant through seeds cannot meet the demand for the required number of plants, as production is limited. This situation has resulted in a sudden increase in demand for *C. spinosa* L. (Polat, 2007; Chalak and Elbitar, 2006). The aerial parts of the plant are rich in polyphenols or flavonoids, which are used for their antifungal and anti-inflammatory properties (Zhou *et al*., 2011; Al-Snafi., 2015). Additionally, it contains rutin as a flavonoid (Zhou *et al*., 2010; Argentieri *et al*., 2012). Al-Snafi (2015) indicated that this species is rich in bioactive compounds such as flavonoids, glucosinolates, phenolic acids, and alkaloids used for medicinal, culinary, and ornamental purposes. Furthermore, various health-promoting properties of caper extracts have been reported, such as anti-cancer and antioxidant activities (Argentieri *et al*., 2012; Moghaddasi *et al*., 2012; Miransari and Smith, 2014; Atemni *et al*., 2021).

The application of plants in the handling of infection is an ancient technique used for treating bacteria and fungi (Obeidat *et al*., 2011; Ibrahim, 2012; Shatnawi *et al*., 2021a). Many previous investigations have indicated that medicinal plants, such as *C. spinosa* L., are known to be an important source of bioactive natural products, such as phenols and flavonoids (Mahboubi and Mahboubi, 2014; Eid *et al*., 2023; Koufan *et al*., 2022). These plants contain different compounds with potentially significant therapeutic applications against various bacteria and fungi (Tlili *et al*., 2010; Gull *et al*., 2015; Alrayes *et al*., 2018, Shatnawi *et al*., 2021b). Moreover, despite the availability of numerous antimicrobial drugs, their use is hindered by factors such as low potency, poor solubility, and the emergence of resistance among microorganisms (Tlili *et al*., 2011; Adwan and Omar, 2021). Therefore, the search for the detection of new antimicrobial plant material is essential and stimulates the study of new chemotherapeutic agents in *C. spinosa* L. (Gull *et al*., 2015). *C. spinosa* L. stands out as a promising medicinal plant with a wide range of pharmacological activities that can be utilized in various medical applications due to its effectiveness and safety (Issah and Duran, 2021; Shatnawi *et al*., 2021a; AlMousa *et al*., 2022).

The whitefly (*Bemisia tabaci* Gennadius) is one of the main pests affecting vegetables in Jordan, leading to significant losses in plant production. It also spreads viruses such as the TYLCV virus (Gangwar *et al*., 2018) and can cause harm to plants through direct feeding, thereby increasing crop loss. The extensive use of costly chemical pesticides to control insects poses a threat to humans, as insects gradually develop resistance to these chemicals over time. Additionally, chemical pesticides can adversely affect non-target beneficial organisms. Medicinal plant extracts present an attractive alternative to chemical insecticides (Cruz-Estrada *et al*., 2013). They are considered as safe with no negative impact on the environment (Solanki *et al*., 2018; Taggar *et al.,* 2020).

In this study, *C. spinosa* L. was assessed for its potential as a green pesticide using different extraction solvents to preserve the main compounds in plant extracts. *In vitro* propagation offers several advantages over traditional propagation methods, such as the production of uniform plants throughout the year (Al Shhab *et al*., 2022; Shatnawi *et al*., 2022). To our knowledge, there are few studies related to caper native to Jordan concerning in vitro propagation, antimicrobial activity, and no prior investigations into insecticidal activity. Furthermore, *in vitro* propagation can overcome the limitations of conventional methods. Therefore, this study initiated in vitro protocols for *C. spinosa* L. under controlled conditions Additionally, we evaluated the potential antimicrobial activities (i.e., antibacterial and antifungal) and insecticidal activities of plant extracts from *in vitro*, *ex vitro*, and callus cultures of *C. spinosa* L. plants.

Materials and Methods

Plant material and culture conditions

Seeds of *C. spinosa* L. were collected from Malka, Irbid (32.6734° N and 35.7517° E). The experiment was conducted at the Plant Tissue Culture Laboratory, Faculty of Agricultural Technology, at Al-Balqa Applied University, Al-Salt, Jordan. The seeds were surface disinfected under a laminar flow cabinet using a 4% sodium hypochlorite (Clorox) solution. Then, the seeds were washed under running tap water for 2 minutes. After that, the seeds were shaken in a Clorox solution containing two drops of Tween 20. Seeds were washed with 70% ethanol for one minute, and then rinsed three times with sterile distilled water for four to five minutes each time. Seeds were germinated *in vitro* on water media (water and 8 g/L agar agar) supplemented with 30.0 g/L sucrose and 8 g/L agar agar. After seed germination *in vitro*, the new microshoots were subcultured on MS medium-free hormone at 24 ± 2 °C under 16 h light and 8 h dark and incubated in the growth chambers with photosynthetic photon flux density (PPFD) of 50 μmol cm^{-2} s^{-1} provided by cool white fluorescent lamps. Microshoots were then subcultured onto a 250 mL Duran flask containing 80 ml MS medium supplemented with 30 g/L sucrose and 0.01 mg/L benzyl amino purine (BAP). The microshoots were subcultured every 5-7 weeks and kept under the same conditions, which were mentioned previously to initiate sufficient plant material before experiment initiation.

In vitro shoot multiplication

Microshoots were proliferated as reported by Shatnawi (2011b). Explants were subcultured on the MS (Murashige and Skoog, 1962) medium-free hormone. Microshoots 15 mm in length with apical meristem were then cultivated on fresh MS medium. For shoot proliferation, the microshoots were cultured on MS medium enriched with Benzyl amino purine (BAP) and thidiazuron (TDZ) at various concentrations (0.0, 0.5, 1.0, 1.5, and 2.0 mg/L). Each treatment consisted of ten replicates and each sample contained three microshoots. Microshoots were incubated under the growth room conditions described above and data were collected on the number of new shoots per microshoot, shoot height in addition to fresh and dry weights after five weeks.

Callus induction

Leaves from *in vitro* shoots (2-3 mm) were placed Petri dish containing 10 ml of MS, at 24 ± 2 °C in the dark in a growth room, which was added with different concentrations of benzyl amino purine (BAP), 2,4-dichloro phenoxy acetic acid (2,4-D) and naphthalene acetic acid (NAA) at 0.0, 1.0, 2.0 mg/L.

Callus growth

Initiated calli were subcultured on 20 ml solid MS medium containing 1-2 mg/L 2, 4-D, and 30 g/L sucrose. The calli (250 mg approximately) were transferred to fresh MS media supplemented with different concentrations of benzyl amino purine (BAP), 2,4-dichloro phenoxy acetic acid (2,4-D) and naphthalene acetic

acid (NAA) at 0.0, 1.0, 2.0 mg/L. Each treatment consisted of five completely randomized replicates and each replicate contained four samples. Data were collected after thirty days on callus fresh weight, texture, and color. Callus dry weights were recorded. Each treatment consisted of five replicates and each replicate contained four samples.

Antimicrobial activity

Tested plants

Ex-vitro, *in vitro,* and callus cultures were obtained for this study from the tissue culture laboratory at Al-Balqa Applied University.

Tested bacterial species

In the current study, six bacterial species were used: four Gram-positive bacteria (*Micrococcus latus, Staphylococcus epidermidis, Staphylococcus aureus*, and *Bacillus cereus,)* and two Gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*). These bacteria species were obtained from the laboratory of microbiology, at Yarmouk University, Jordan.

Tested fungal species

In the current study, four fungal species were used: *Aspergillus niger mutant black, Penicillium italicum, Penicillium chrysogenum* and *Aspergillus nidulans*. These species were obtained from the Laboratory of Fungal Genetics, Mu'tah University, Jordan.

Fungal and bacterial growth media

Bacteria and fungi strains were cultivated on nutrient agar (NA; Fluka, Germany) and potato dextrose agar (PDA; Himedia, India) respectively. The media (about 15 ml) were dispensed into 9 cm sterile Petri dishes to obtain 0.4 cm thickness.

Extraction of plant compounds

Twenty grams of *ex vitro*, *in vitro,* and callus of *C. sipnosa* L. was dried out at room temperature in the shade for five days, then ground to a fine powder, then extracted with 100 ml ethanol or methanol, by soaking for one week (Ndukwe *et al*., 2006). The solvents were detached using a rotary evaporator (HeidolphVV2000, Germany) under reduced pressure at temperatures below 50 °C. The resulting crude extracts were stored at -20 °C until used. Stock solutions of extracts were assembled in dimethylsulphoxide (DMSO) (Ambrozin *et al*., 2004) at 250 µg/µl concentration, and then two volumes (40 µl and 80 µl) were tested against the microbe. Positive control bactericide (oxytetracycline), fungicide (cyclohexamine), and negative (DMSO) controls were attained. The DMSO was used at the same volume as the extracts. To test the ability of plant extract to inhibit bacteria, and insects (*Bemisia tabaci*) extracts were dissolved in DMSO.

Antibacterial activity assay by the agar well diffusion method

The bacterial species were spread on nutrient agar plates with sterile swap. Plates were divided into 4 quadrates. *C. spinosa* L. extract aliquots (40 µl and 80 µl) at 250 µg/µl concentration were added to wells and left for one hour to diffuse, then plates were incubated at 37 °C for 24 h. The antibiotic Oxytetracycline was used as a positive control and prepared in 250 µg/µl and their antibacterial activity was tested in the same manner, also the solvent dimethyl sulfoxide (DMSO) was tested as a negative control, and the zones of inhibition were measured in mm at the end of the incubation period. The growth of the microbe was determined by measuring the zone of inhibition, which was compared with a standard antibiotic (oxytetracycline).

Antifungal activity assay by the agar well diffusion method

An aliquot of 100 µl spore suspension (1×10^8 spores/ml) of each isolate was streaked in radial patterns on the surface of complete media plates. *C. spinosa* L. extract aliquots (40 and 80 µl) at 250 µg/µl concentration were added into wells and left for one hour to diffuse, and then the plates were incubated at 30 °C for 48 h. The antifungal compound (Cyclohexamine: positive control), the dimethyl sulfoxide (DMSO) was tested as negative control was prepared in 250 µg/µl and their antifungal activity was tested in the same manner. The zones of inhibition were measured in mm at the end of the incubation period. Microbial growth was determined by measuring the diameter of the zone of inhibition and then it was compared with standard antifungal (Cyclohexamine).

Insecticidal activity

The insecticidal activity of *C. spinosa* L. was evaluated against the sweet potato whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleurodidae). Plant extracts were tested on the 4th nymphal instar. The effects of plant extracts on the sweet potato whitefly were studied under laboratory conditions. A simple cage was prepared from one 9 cm Petri plate. Four mm holes were made through the upper plate cover. Each plate contained a slide and for each slide, ten drops of 10 µl extract were made by using a micropipette. To reduce the evaporation of extract under each slide a wet filter paper was placed at the bottom of the plate. 4th nymphal instars were gently collected using special needle tools for bee grafting methods, nymphs were collected from cotton-infected plant leaves, and each nymph was added to the extract drop under the dissecting microscope to ensure accuracy and prevent nymph rupture. Moreover, plant extraction was prepared at 3 different concentrations, 1%, 5%, and 10% using DMSO and by using two different ex-plant (*in vitro* and *ex vitro*). DMSO was set as a negative control, each treatment consists of 5 replicates and each replicate consists of 10 nymphs. The cages were kept at 24 °C ±2 and a 16 hr photoperiod, Data were collected after, one, two, and three days. Colour change and rupture of the whitefly nymph indicated death of the tested insect, development of nymph to adult was considered survival.

Statistical analysis

A completely randomized design (CRD) was used in this study. The gotten results were exposed to a one-way ANOVA test. Each treatment had 10 replicates and each experiment was repeated three times. Post hoc multiple comparisons using the Tukey Honestly Significant Difference (HSD) test were used at a 0.05 probability level. Data were analyzed using SPSS programs version 17 (SPSS, 2017).

Results and Discussion

Effect of cytokinins on shoot proliferation

The main target of this study was to develop effective *in vitro* propagation, callus induction, and antimicrobial and insecticidal activity for *in vitro C. spinosa* L. plant. Supplements of various concentrations of BAP and TDZ significantly increased the number of new shoots (Table 1). At all tested concentrations, the average number of shoots developed per explant was higher than that of the control (Table 1). *C. spinosa in vitro* culture showed a high proliferation on MS medium added with 0.5 mg/L BAP (5.9 microshoot/explants). BAP produces higher shoot proliferation than TDZ (Table 1). While the medium containing 0.5 mg/L TDZ, an average number of shoots per plant (4.7) was initiated with an average shoot length of 21.5 mm. Moreover, microshoot development was influenced by plant hormones (Shatnawi *et al*., 2011b). MS medium added with TDZ produced rosette shoots with small leaves that were rather long (Table 1).

Of the two cytokinins tested, BAP was verified to enhance microshoots rather than TDZ. However, BAP at 0.5 mg/L produced the maximum number of shoots but their size significantly reduced. This may be

due to the suppression of apical dominance that leads to the production of more shoots and reduced shoot length. Shatnawi *et al*. (2011a) got a similar result on *Stevia rebaudiana*. BAP has been also used to induce multiple shoots in *Artemisia vulgaris* L. (Sujatha *et al*., 2008). Similarly, Islam *et al*. (2005) found that BAP assembled the maximum number of shoots compared with other growth regulators. In contrast, Abdellatef *et al*. (2010) found that using a combination of BAP and NAA produced a maximum shoot induction rate. Although TDZ has been shown to increase shoot production, many previous reports have demonstrated that TDZ does not benefit or hinder shoot production and elongation (Kartsonas and Papafotiou, 2007).

Table 1. Effect of different concentrations of BAP and TDZ on the number of axillary shoots, maximum shoot length, and callus formation of *in vitro* grown *C. spinosa* L. after six weeks of growth periods (mean values ± standard deviation)

Concentration (mg/L)	Number of axillary shoot/ explants	Shoot length (mm)	Callusing (%)
0.0	2.3 ± 0.4 a	20.4 ± 1.8 de	20%
BAP			
0.5	5.9 ± 0.7 c	18.9 ± 0.5 cde	50%
1.0	4.5 ± 0.5 bc	16.9 ± 1.2 bc	100%
1.5	4.5 ± 0.5 bc	17.5 ± 1.3 bcd	100%
2.0	4.1 ± 0.4 bc	18.5 ± 0.5 cde	100%
TDZ			
0.5	4.7 ± 0.8 bc	21.5 ± 1.0 e	100%
1.0	4.8 ± 0.6 bc	15.7 ± 1.1 abc	100%
1.5	3.7± 0.3 b	14.4 ± 0.9 ab	100%
2.0	3.6 ± 0.3 b	12.9 ± 0.6 a	100%

"Means within columns having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

Callus growth

This experiment tests the effect of various growth regulators and concentrations on callus production and growth over three months. Initial callus induction occurred on the cut surface of the explants. Callus was induced after one month using BAP, 2,4-D, and NAA at 1.0 mg/L (Table 2). On the other hand, medium supplemented with 2.0 mg/L BAP, 2,4-D and NAA increased callus production. After two months, a maximum callus induction of about 33.1 mm was obtained on MS medium supplemented with 2.0 mg/L NAA. NAA improved the growth of the callus after one month. However, after three months, callus production increased significantly. The callus induced in medium supplemented with BAP was dark brown. On a medium added with NAA or 2,4-D calli were formed from the explants after 2 months in growth media (Table 2). Maximum callus development was observed on MS medium supplemented with 2.0 mg/L NAA. Al-Ajlouni *et al*. (2012) were able to form the callus of *Hordeum vulgare* on MS media added with 2,4-D. Also, this result is similar to previous findings on *Oryza sativa* (Abe and Futsuhara, 1986).

Table 2. Effect of time and different growth regulator concentrations on callus diameter, fresh weight, and dry weight of *in vitro* grown *C. spinosa* L (mean values ± standard deviation)

Concentration (mg/L)	1st month size (mm)	2nd month size (mm)	3rd month size (mm)	Fresh weight (mg)	Dry weight (mg)
0.0	0.00 ± 0.00 a	3.00 ± 1.39 a	4.06 ± 1.89 a	1068 ± 348.3 a	90.67 ± 11.40 a
1.0 BAP	0.00 ± 0.00 a	4.37 ± 1.51 a	6.43 ± 1.88 a	4675 ± 2482 ab	326.7 ± 166.4 bc
1.0 2,4D	7.18 ± 0.95 b	16.0 ± 1.50 b	19.2 ± 1.50 b	2651 ± 34.92 a	153.6 ± 28.89 b
1.0 NAA	11.9 ± 0.72 d	19.7 ± 1.03 b	23.9 ± 1.06 c	4752 ± 968.3 ab	374.7 ± 43.23 bc
2.0 BAP	9.13 ± 0.73 c	18.6 ± 1.21 b	23.5 ± 1.34 c	4668 ± 967.1 ab	361.1 ± 42.99 bc
2.0 2,4D	9.10 ± 0.85 c	24.4 ± 1.73 c	29.1 ± 1.72 d	6507 ± 987.0 b	369.6 ± 61.96 bc
2.0 NAA	12.5 ± 0.64 d	33.1 ± 1.21 d	37.6 ± 1.31 e	8042 ± 789.0 b	527.5 ± 30.50 c

"Means within columns having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

Antimicrobial activity

Methanolic extracts from *ex vitro*, *in vitro,* and callus exhibited diverse significant activities as opposed to the tested bacteria. Most of methanolic extracts showed antimicrobial activity against gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus, Micrococcus latus* species and gram-negative bacteria such as *Escherichia coli*, and *Salmonella typhimurium*. Maximum *ex vitro* plant antimicrobial activity was (24±0.58 mm) against *Staphylococcus epidermidis* (Table 3). Callus extracts did not show any inhibitory effect on *Salmonella typhimurium* and *Micrococcus latus*. Moreover, methanolic *in vitro* crude extract did not show any prevention activity for *Escherichia coli* and *Staphylococcus epidermidis.* Callus extract displayed intrinsic antibacterial properties. Of the six microorganisms used, *Escherichia coli* and *Bacillus cereus* were the most sensitive to callus methanolic extract. At both concentrations, the methanolic extracts had inhibitory effects on *Staphylococcus aureus*, *Bacillus cereus, Micrococcus latus, Escherichia coli*, *Staphylococcus epidermidis,* and *Salmonella typhimurium* (Table 3).

Table 3. Antibacterial activity of *C. spinosa* L. using methanolic plant extract (mean values ± standard deviation)

Strain	Crude amount (µl)	Inhibition zone (mm) in different solvent system				
		Methanol			Control	
		Ex-vitro	*In vitro*	callus	Positive oxytetracycline	Negative DMSO
Staphylococcus aureus	40	19.0 ± 0.58 b	13.7 ± 0.33 c	11.0 ± 0.58 d	35.3 ± 0.88 c	0.00±0.00 a
	80	24.0 ± 0.58 a	15.0 ± 0.58 c	16.0 ± 0.58 c	42.3 ± 0.88 a	0.00±0.00 a
Micrococcus latus	40	18.0 ± 0.58 bc	12.0 ± 0.58 cd	0.00 ± 0.00 e	35.0 ± 1.15 c	0.00±0.00 a
	80	24.0 ± 0.58 a	13.0 ± 0.58 c	0.00 ± 0.00 e	44.3 ± 1.45 a	0.00±0.00 a
Escherichia coli	40	17.0 ± 0.58 bc	0.00 ± 0.00 e	23.0 ± 0.58 b	38.0 ± 0.58 b	0.00±0.00 a
	80	20.0 ± 0.58 b	0.00 ± 0.00 e	26.0 ± 0.58 a	42.0 ± 1.15 a	0.00±0.00 a
Bacillus cereus	40	16.0 ± 0.58 c	10.0 ± 0.58 d	23.0 ± 0.58 b	20.7 ± 0.88 d	0.00±0.00 a
	80	17.0 ± 0.59 bc	10.0 ± 0.58 d	26.0 ± 0.58 a	38.3 ± 1.20 b	0.00±0.00 a
Staphylococcus epidermidis	40	19.0 ± 0.58 b	0.00 ± 0.00 e	16.0 ± 0.58 c	35.0 ± 1.15 c	0.00±0.00 a
	80	25.0 ± 0.58 a	0.00 ± 0.00 e	19.0 ± 0.58 cb	45.0 ± 1.52 a	0.00±0.00 a
Salmonella typhimurium	40	20.0 ± 0.58 b	19.0 ± 0.58 b	0.00 ± 0.00 e	21.7 ± 0.88 d	0.00±0.00 a
	80	22.0 ± 0.58 ab	20.0 ± 0.58 a	0.00 ± 0.00 e	40.3 ± 0.88 b	0.00±0.00 a

"Means within columns having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

Ethanolic extracts of *ex vitro*, *in vitro,* and callus in this study demonstrated a broad spectrum of activity in *Staphylococcus aureus*, *Bacillus cereus, Micrococcus latus, and Staphylococcus epidermidis, Escherichia coli* and *Salmonella typhimurium* (Table 4). Callus ethanolic extract did not show antibacterial activity against *Staphylococcus epidermidis* and *Salmonella typhimurium*. On the other hand, callus displayed intrinsic antibacterial properties against *Staphylococcus aureus*, *Bacillus cereus, Micrococcus latus,* and *Escherichia coli*. Ethanolic *ex vitro* and *in vitro* extracts demonstrated antimicrobial activity against all tested bacteria. The highest activity (26 mm) diameter of the inhibition zone was against *Staphylococcus epidermidis* with 80 µl crude extract.

The attained investigation exhibited that ethanol and methanol extracts of *C. spinosa* L varied in antibacterial and antifungal activities. Lemberkovics *et al.* (2003) showed that the constitution of plants, altered by the method of extraction. Results of this study have shown that *C. spinosa* L. has a high possibility of antimicrobial influences. Plant extract having active compounds could pass through the cell wall of the microorganism, which may impede it is growth (Obeidat *et al*., 2011). The investigated plants showed reasonable antibacterial activity. The active compound(s) may exist in inadequate amounts in the crude extracts (Shatnawi *et al*., 2021 a, b). Alternatively, if the active chemical exists in elevated quantities, there could be an opposing influence (Jager *et al*., 1996). With no antibacterial activity, extracts may be active against other bacterial species that were not tested (Shale *et al*., 1999).

Table 4. Antibacterial activity of *C. spinosa* L. using ethanolic plant types extract (mean values ± standard deviation)

Strain	Crude amount (µl)	Inhibition zone (mm) in different solvent system				
		Ethanol			Control	
		Ex-vitro	***In vitro***	Callus	Positive oxytetracycline	Negative DMSO
Staphylococcus aureus	40	16.0 ± 0.58 d	13.0 ± 0.58 c	14.0 ± 0.58 c	35.3 ± 0.88 b	0.00±0.00 a
	80	19.0 ± 0.58 cd	16.0 ± 0.58 b	20.0 ± 0.58 a	42.3 ± 0.88 a	0.00±0.00 a
Micrococcus latus	40	20.0 ± 0.58 c	9.00 ± 0.58 d	0.00 ± 0.00 d	33.0 ± 1.15 b	0.00±0.00 a
	80	22.6 ± 0.67 b	9.00 ± 0.58 d	13.6 ± 0.33 c	44.3 ± 1.45 a	0.00±0.00 a
Escherichia coli	40	16.0 ± 0.58 d	11.00 ± 0.58 cd	13.3 ± 0.33 c	36.0 ± 0.58 b	0.00±0.00 a
	80	20.0 ± 1.00 c	11.3 ± 0.33 cd	17.3 ± 0.88 b	42.0 ± 1.15 a	0.00±0.00 a
Bacillus cereus	40	22.3 ± 0.33 b	11.0 ± 0.58 cd	14.7 ± 0.67 c	22.7 ± 0.88 c	0.00±0.00 a
	80	18.0 ± 0.58 cd	19.0 ± 0.58 a	15.7 ± 0.33 cb	40.3 ± 1.20 a	0.00±0.00 a
Staphylococcus epidermidis	40	19.3 ± 0.67 cd	12.7 ± 0.33 c	0.00 ± 0.00 d	35.0 ± 1.15 b	0.00±0.00 a
	80	26.7 ± 0.67 a	16.0 ± 0.58 b	0.00 ± 0.00 d	45.0 ± 1.52 a	0.00±0.00 a
Salmonella typhimurium	40	20.3 ± 0.33 c	15.0 ± 0.58 b	0.00 ± 0.00 d	21.7 ± 0.88 c	0.00±0.00 a
	80	21.9 ± 0.33 bc	19.3 ± 0.33 a	0.00 ± 0.00 d	40.3 ± 0.88 a	0.00±0.00 a

"Means within columns having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

Among the plant extracts, *ex vitro* extract exhibited high inhibition of fungal development. The *in vitro* methanolic extract was found to be effective for all fungi including *Penicillium chrysogenum*, *Penicillium italicum,* and *Aspergillus nidulans* (Table 5). Maximum inhibition with methanolic callus extract was reported against *Penicillium italicum* and *Aspergillus nidulans* (Table 5).

Table 5. Antifungal activity of *C. spinosa* L. using methanolic plant types extract (mean values ± standard deviation)

Strain	Crude amount (µl)	Inhibition zone (mm) in different solvent system				
		Methanol			Control	
		Ex-vitro	***In vitro***	callus	Positive cyclohexamine	Negative DMSO
Penicillium italicum	40	18.0 ± 1.15 c	13.0 ± 0.58 c	20.3 ± 0.88 b	27.0 ± 0.58 c	0.00±0.00 a
	80	26.0 ± 1.15 b	17.7 ± 0.88 b	23.0 ± 0.58 a	32.0 ± 0.58 b	0.00±0.00 a
Penicillium chrysogenum	40	25.7 ± 0.88 b	12.0 ± 1.53 c	0.00 ± 0.00 c	31.3 ± 0.88 b	0.00±0.00 a
	80	30.7 ± 1.45 a	20.7 ± 0.88 a	0.00 ± 0.00 c	40.0 ± 0.58 a	0.00±0.00 a
Aspergillus nidulans	40	20.0 ± 1.15 c	17.0 ± 1.15 b	20.0 ± 0.58 b	33.3 ± 0.88 b	0.00±0.00 a
	80	23.7 ± 0.67 bc	21.3 ± 0.88 a	23.0 ± 0.58 a	42.0 ± 1.15 a	0.00±0.00 a
Aspergillus niger mutant. Black	40	10.00 ± 1.15 d	11.00 ± 0.58 d	0.00 ± 0.00 c	24.3 ± 1.45 d	0.00±0.00 a
	80	22.0 ± 2.89 bc	22.0 ± 1.53 a	0.00 ± 0.00 c	40.0 ± 0.58 a	0.00±0.00 a

"Means within columns (for each preservation period) having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

The antifungal activities showed by *ex vitro, in vitro* and callus were similar. Analysis of the data revealed that among the tested extracts, the *ex vitro* extract revealed the maximum rates of antifungal activeness (Table 6). It showed antifungal activity against *Penicillium italicum* (25 mm), *Penicillium chrysogenum* (22.7) mm, *Aspergillus nidulans* (23 mm). The ethanolic extract of callus possessed an antifungal effect on *Penicillium italicum*, and *Aspergillus nidulans*. Moreover, callus extracts showed no activity against *Penicillium chrysogenum* and *Aspergillus niger mutant black.* The inhibitory effects of all the extracts tested against fungi increased with higher levels. The plant extract was very effective against some fungal species. The antifungal activities of ethanolic extract compared with positive control (Cyclohexamine) were similar (Table 5 and 6).

Table 6. Antifungal activity of *C. spinosa* L. using ethanolic plant types extract (mean values ± standard deviation)

Strain	Crude amount (µl)	Inhibition zone (mm) in different solvent system				
		Ethanol			Control	
		Ex-vitro	***In vitro***	callus	Positive cyclohexamine	Negative DMSO
Penicillium italicum	40	17.0 ± 0.58 c	0.00 ± 0.00 d	20.0 ± 0.58 c	27.0 ± 0.58 c	0.00±0.00 a
	80	25.0 ± 1.53 a	16.7 ± 0.88 a	23.0 ± 0.58 b	32.0 ± 0.58 b	0.00±0.00 a
Penicillium chrysogenum	40	19.0 ± 0.58 cb	0.00 ± 0.00 d	0.00 ± 0.00 e	33.3 ± 0.88 b	0.00±0.00 a
	80	22.7 ± 0.88 b	16.0 ± 0.58 a	0.00 ± 0.00 e	40.0 ± 0.58 a	0.00±0.00 a
Aspergillus nidulans	40	17.7 ± 0.67 d	9.00 ± 0.58 c	25.0 ± 0.58 b	33.3 ± 0.88 b	0.00±0.00 a
	80	23.0 ± 0.58 b	14.0 ± 2.52 b	33.0 ± 0.58 a	42.0 ± 1.15 a	0.00±0.00 a
Aspergillus niger mutant. Black	40	10.00 ± 0.58 e	0.00 ± 0.00 d	0.00 ± 0.00 e	24.3 ± 1.45 d	0.00±0.00 a
	80	21.0 ± 2.10 b	0.00 ± 0.00 d	0.00 ± 0.00 e	40.0 ± 0.58 a	0.00±0.00 a

"Means within columns (for each preservation period) having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

Insecticidal activity of C. spinosa L. extract

In the present study, the effect of *C. spinosa* L. methanolic and ethanolic extracts was investigated for their insecticidal activities against the fourth nymphal stage of the sweet potato whitefly (*B. tabaci)* (Table 7). Results showed that *B. tabaci* 4th nymphal instar exposed to *C. spinosa* L. extracts suffered mortality ranging from 2 to 28%. In most instances, both ethanolic and methanolic extracts affected the survival of *B. tabaci*

more than the control. After three days of exposure, the highest mortality (28%) was achieved when *B. tabaci* was treated with ethanolic extract at a concentration of 5%, while the lowest mortality (7.5%) was observed with methanolic extract at 1% concentration. There is a trend of increased mortality with increasing the concentration of the extract. However, no significant differences were found due to concentration or type of solvent. Therefore, *in vitro,* ethanolic extract at a concentration of 5% was found to be the most effective against the pest after three-day incubation periods.

Table 7. Mortality percentage of *B. tabaci* nymphs treated with methanolic and ethanolic extracts from *in vitro* and *ex vitro C. spinosa* L. plant (mean values ± standard deviation)

Plant	Solvent	Concentration	Day 1	Day 2	Day 3
In vitro	Methanol	1%	13.0 ± 5.83 a	13.0 ± 5.83 e	22.0 ± 11.0 b
		5%	11.0 ± 4.47 b	20.0 ± 4.89 b	22.0 ± 5.10 b
		10%	7.00 ± 2.45 c	15.0 ± 6.00 d	17.0 ± 7.48 d
	Ethanol	1%	5.00 ± 2.45 d	5.00 ± 2.45 h	17.0 ± 7.48 d
		5%	2.00 ± 2.00 e	23.0 ± 9.69 a	25.0 ± 8.00 a
		10%	5.00 ± 2.45 d	12.0 ± 3.74 e	22.0 ± 8.37 b
Ex vitro	Methanol	1%	2.50 ± 2.00 e	7.00 ± 4.00 g	7.50 ± 4.00 g
		5%	7.00 ± 4.00 c	9.00 ± 3.74 f	24.0 ± 10.7 a
		10%	9.00 ± 3.74 c	13.0 ± 2.00 e	19.0 ± 4.89 c
	Ethanol	1%	13.0 ± 3.74 a	18.0 ± 5.09 c	22.0 ± 4.47 b
		5%	11.0 ± 7.75 b	11.0 ± 7.75 f	22.0 ± 7.07 b
		10%	11.0 ± 4.47 b	15.0 ± 7.48 d	15.0 ± 7.48 e
-ve control (DMSO)			7.00 ± 4.00 c	11.0 ± 3.16 f	13.0 ± 3.74 f

"Means within columns having different letters are significantly different according to Tukey"s Honestly Significant Difference (HSD) test was used at 0.05 probability level".

The present study revealed the effect of *C. spinosa* L. extract on *B. tabaci.* The significant difference in insecticidal activity against *B. tabaci* nymph was observed with crude methanolic and ethanolic extract from *C. spinosa* L. in comparison with time, but not with solvent concentration and solvent type. This study showed that *ex-vitro* and *in vitro* plant extracts were moderately active because they caused over 20-28% nymph mortality with the use of respective plant extracts (Table 7). Additionally, plant extracts are safer for humans, beneficial organisms, and the environment than traditional insecticides (Ateyyat *et al*., 2009; Derwich *et al*., 2009). Kazem and Farghaly, (2009) found that the best treatment against whitefly adults was detected with a 2.5% concentration extracted from capsicum and ginger. In addition, Ateyyat *et al.* (2009) reported that the treatment with *C. spinosa* L. extract caused the death of the nymph of *B. tabaci* by 55% percentage.

Conclusions

An accessible and constant method for clonal proliferation and callus induction was recognized. *C. spinosa* L. *in vitro* culture influenced in MS medium regulated by 0.5 mg/L BAP (5.9 microshoot/explants). Callus was obtained on MS medium supplemented with 2.0 mg/L NAA. Caper plant is an excellent candidate plant that could be involved in the drug industry as an antimicrobial agent. *Ex vitro*, *in vitro* plantlets, and callus of *C. spinosa* L. confirmed a wide range of activity in contradiction of both gram-positive and gram-negative bacteria and fungi. Bioactive substances from this plant can therefore employed for the treatment of various bacteria and fungi. Additionally, *C. spinosa* L. plants possess good insecticidal activity based on the result of this study. Moreover, plant extracts from *ex vitro* and *in vitro* showed that *B. tabaci* 4th nymphal instar exposed to *C. spinosa* L. extracts suffered mortality ranging from 2-28% nymph mortality with the use of respective plant

extracts. *C. spinosa* L. is highly recommended to propagate in huge amounts through *in vitro* propagation to meet its demand for different purposes and enhance the sustainability of this plant. Finally, *C. spinosa* L. could be used as a health remedy in folklore medication, and possesses good insecticidal activity and concluded to include this plant species in pesticide synthesis as a green pesticide against white flies due to the presence of many chemicals in *C. spinosa* L. plants.

Authors' Contributions

WS mentored all experimental work, plant extract, tissue culture, and writing of the manuscript; MS tissue culture experiment and writing the manuscript; MA insect experiment and statistical analysis; RS callus experiment and writing manuscript; TA microbial experiment; MM fungi experiment and writing the manuscript; ARA microbial experiment and collected data**;** AA experimental technical work and collecting data.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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