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PHYTOTOXICITY AND ANTIFUNGAL ACTIVITY OF CAPPARIS SPINOSA L.

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Abstract:-

In laboratory bioassays, this study evaluated the phytotoxic potential of Capparis spinosa organs (leaves, fruits and roots) aqueous extracts on germination and seedlings growth of Triticum aestavium and Raphanus sativus. The results suggested that C. spinosa leaf, fruit and roots aqueous extract appeared to have phytotoxic effect on the recipient species compared to control. The germination percentage and all growth parameters of T. aestavium and R. sativus were significantly reduced gradually with the increase of aqueous extract concentration levels. However the reduction was varied and could be parts of the donor species and extract concentration dependent. This study also investigated the antifungal activities of three different solvents (ethanol, methanol and acetone) extracts of C. spinosa organs, these extracts were tested for their toxicity in vitro against Aspergillus niger, Penicillium sp. and Trichoderma viride at concentrations 10% (w/v). All C. spinosa organs extracts had different degrees of antifungal activity against the tested fungi, the highest antifungal activity was recorded for fruit ethanolic extract against Trichoderma whereas, acetone extract was the least effective extract against the tested fungi. Generally, tested fungi Trichoderma and Aspergillus were found to be more sensitive to C. spinosa organs extracts while Penicillium showed high resistance. The C. spinosa fruit ethanolic extract may be recommended as a potent bio-fungicide. Extensive studies should be undertaken for the ethanolic extract of C. spinosa organs as a strong antifungal agent against fungal plant diseases.

Keywords:- *Phytotoxicity, Capparis spinosa , Triticum aestavium ,Raphanus sativus, Antifungal activity.*

INTRODUCTION

Allelopathy is a natural ecological phenomenon in which different organisms affect the functioning of other organisms in their vicinity, negatively or positively by releasing secondary metabolites [1]. The main principle in allelopathy arises from the fact that plants produce thousands of chemicals; and many of these chemicals are released by leaching, exudation, or decomposition processes. Subsequently, some of these compounds which are known as allele-chemicals alter the growth or physiological functions of recipient species [2].

Plants or organisms that release these compounds are called 'donor species', while those that are influenced in their growth and development are called 'target or recipient species'. Research conducted in the last half of the twentieth century demonstrated growth inhibition by allele-chemicals that influenced vegetation patterns, rate and sequences in plant succession, weed abundance, crop productivity, and problems in replanting fruit and other crops [3]. Plant protection is effective but rather costly and problematic due to environmental pollution, in the past two decades, much more work has been done on plant derived compounds as environmentally safe alternatives to herbicides for the weed control [4]. In this regard, the use of plant species having allelopathic properties can reduce the dependency on synthetic herbicides and increase crop yields [5]. For studies with plant extracts, allelochemicals isolated from plant tissue, collected from exudates or even synthetic compounds identical to natural ones, it was established the term ''phytotoxicity'' to distinguish allelopathy (as a phenomenon occurring in natural environment) from studies conducted in laboratory [6]. *Capparis spinosa* is a flowering plant of the family capparidaceae; it is a common perennial shrub growing both wild and cultivated. The plant has a natural distribution in the coastal regions of the entire Mediterranean Sea basin. It grows in poor soils especially in dry areas, thus playing an environmental role in fixing soils and limiting erosion. Different organs of *C. spinosa* including young shoots, flower buds, fruits, leaves and seeds have been employed in drugs, foods and cosmetics [7]. Petroleum ether, methanol, hexane, ethanol and aqueous crude extracts of the whole aerial parts of *Capparis spinosa* exhibited variable degrees of antimicrobial activities, including antifungal, antibacterial and antiviral activities [8]. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. Nowadays, a commercial pesticide used against plant diseases is found to cause damage to environment and human health. Because of that conducting a research of alternative control methods comes into prominence for minimizing used commercial pesticide. Research found that compounds in the structure of plants and essential oil were showed antifungal, antibacterial, insecticidal, nematicidal, herbicidal and antiviral activities [9].The antifungal activities of ethanolic extract of (*Capparis spinosa* L.) was investigated *in vitro* against *Alternaria alternata, Fusarium oxysporum, Phoma destructiva, Rhizoctonia solani,* and *Sclerotium rolfsii* at concentrations of 0, 3, 6, and 9% (v/v), it produced concentration dependent fungal growth inhibition [10]. The phytochemical studies of *Capparis spinosa* extracts showed the presence of many chemical compounds with very interesting biological activities such as alkaloids, fatty acids, phenolic acids, flavonoids, Terpens, vitamins and glycosides [11]. This study aimed to investigate the phytotoxic effects of different *C. spinosa* organs (leaves, fruit and roots) aqueous extracts on two most sensitive plants (*Triticum aestavium*, *Raphanus sativus*) under laboratory conditions. This study also evaluated the antifungal activities of three different solvents (ethanol, methanol and acetone) extracts of *C. spinosa* organs, these extracts were tested for their toxicity and antifungal activity in *vitro* against *Aspergillus niger, Penicillium sp.* and *Trichoderma viride*.

MATERIALS AND METHODS

Plant sampling and preparation of extracts

The fresh samples of leaves, fruits and root were collected from the wild caper (*Capparis spinosa* L.) shrubs growing in the Al-Jabal Al-Akhdhar region - Libya in May 2016. The samples were further identified by taxonomist at the Department of Botany Herbarium, Faculty of Science, and Omar Al-Mukhtar University. The collected samples were washed and dried in the oven at 70^0C for 72 hr and then powdered. Different concentrations of aqueous extract (5, 10, 20 and 40% w/v) were prepared, in addition to the control (distilled water).

Phytotoxic activity

Petri-dish laboratory experiment was applied to investigate the phytotoxic action of donor species aqueous extract (0, 5, 10, 20 and 40%) on germination and seedling growth of the recipient species wheat (*Triticum aestivum* L. Local variety) and radish (*Raphanus sativus* L.). Twenty five seeds of the recipient species were arranged in 9-cm diameter Petri-dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 20-23^{o}C and night temperature from 14-16^{o}C. Five milliliters of each level of the donor species aqueous extract concentrations were added to each replicates. Before sowing, the seeds were surface sterilized by soaking for two minutes in 5% sodium hypochlorite, then, thoroughly washed with tap water for several times followed by distilled water. Treatments were arranged in a completely randomized design with four replications. Seeds were considered germinated when radicle length was 2 mm and the number of germinated seeds was recorded daily, while germination percentage, radicle, plumule length and the dry weight Germination %, plumule and radicle length , seedlings dry weight, seedling vigor index (SVI) and Phytotoxicity % were recorded after 7 days at the end of the experiment.

(SVI) = [seedling length (cm) × germination percentage] [12].
Phytotoxicity % = [(Control -Extracts) / Control] × 100 [13].

Antifungal activity

The plant samples (leaves, fruits and root) were ground to pass 2 mm sieve and the powder plant samples were separately extracted with three different solvents aqueous methanol (methanol: water, 80:20 v/v), aqueous ethanol (ethanol: water, 80:20 v/v) and aqueous acetone (acetone: water, 80:20 v/v). Dry powdered samples of each plant part (10 g) were mixed separately with 100 mL of each solvent for 6 h at room temperature in an orbital shaker. The extract and residues were separated using Whatman's filter paper. The extracts were evaporated and the crude concentrated extracts were stored in refrigerator at 4ºC, until used for further studies. The fungi which tested in this study (*Aspergillus niger* , *Penicillium sp.* and *Trichoderma viride*) obtained from Department of Plant Protection, Faculty of Agriculture, Omar Al- Mokhtar University El- Beyda, Libya. The antifungal activity of the plant extracts was determined using the agar well diffusion method [14], where potato Dextrose Agar (PDA) plates seeded with fungal strain, on each plate wells were made by sterile standard corn borer. Each well was filled with 30µl of the different concentrations of studied plant extracts and the plates were then incubated for 48-72 h at 28°C. Antifungal activity was assessed by measuring the diameter of the growth-inhibition zone (GIZ) in mm for the test organisms comparing to the controls, the results are presented as mean of triplicate. Statistical Analysis

The experimental results were subjected to one way-analysis of variance and the means were separated by the least significant difference, LSD, using Co-stat program.

RESULTS AND DISCUSSION

Phytotoxic activity

The phytotoxic activity of the leaf, root and fruit aqueous extract of *Capparis spinosa* L. on the germination percentage (GP), plumule and radicle length and seedling dry weight of *Raphanus sativus* are represented in Tables 1, 2 and 3 respectively, whereas the phytotoxicity activity of the leaf, root and fruit aqueous extract of *Capparis spinosa* L. on the germination percentage (GP), plumule and radicle length and seedling dry weight of *Triticum aestavium* are represented in Tables 4, 5 and 6 respectively. The data demonstrated that the GP and the growth parameters of the recipient species was significantly affected by applying the different concentrations of the leaf, root and fruit aqueous extract of *Capparis spinosa*. Generally, GP and the growth parameters decreased with the increase in treatment concentrations. Compared to control, reduction increased as the concentration of the donor species increased. The reduction was varied and was parts of the donor species dependent. 40% concentration level had the greatest phytotoxic effect. It had been demonstrated that the main mechanism of allele-chemicals action was the inhibition of specific enzyme activities, hydrolytic enzymes seemed to be one of the most sensitive enzymes to phytotoxic stress, which may decrease activity and hence delay seed germination [15]. Germination of seeds depended on amylase activity that regulated starch breakdown which was necessary for supplying substrates to respiratory metabolism and the release of energy [16]. The higher root growth inhibition with higher extract concentration might be due to the direct contact between the root and phytotoxic substances present in the aqueous extract and the greater permeability of these substances to root tissues than that of shoot tissues. These phytotoxic substances may inhibit cell division which is highly active at meristematic tissue at the growing root tip [17]. In addition, the phytotoxic effect is probably due to the presence of secondary metabolites such as alkaloid, steroids, flavonoids, tannins, phenols, saponin, terpenoids, volatile oils and fatty acids in aqueous extract from aerial parts of *C. spinosa* [18]. Among the three parts of the donor species the leaf aqueous extract had highest phytotoxic effects on germination and other growth parameters; that was followed by root aqueous extract. Least effect was shown by fruit aqueous extract on the recipient species. Highest phytotoxic effect on seedling growth was exhibited by *Capparis spinosa* aqueous extract at 40% concentration; that was followed by 20% concentration (Tables1, 2,3,4,5 and 6).

Table 1: Effect of different concentration levels of *C. spinosa leaf* aqueous extract on germination percentage and some growth parameters of *R. sativus* L.

Conc.	GP%	PT%	RL (cm)	PT%	PL (cm)	PT%	SD wt. (mg/seedlings)	SVI
Control	100	0	6.96 ±3.19	0	4.90 ±1.29	0	0.13 ±0.04	1186
5%	100	0	3.96 ±1.03	43.10	9.38 ±1.53	0	0.04 ±0.02	1334
10%	100	0	1.48 ±1.12	80.67	0.66 ±0.51	86.53	0.03 ±0.02	66
20%	45	55	0.45 ±0.14	93.53	0.50 ±0.08	89.79	0.10 ±0.05	42.75
40%	25	75	0.33 ±0.04	95.25	0	100	0.11 ±0.01	8.25
L.S.D 5%	-	-	1.25	-	2.34	-	0.69	-

GP: germination percentage, PT: Phytotoxic, SVI: seedling vigor index, PL: Plumula length, RL: Radical leng

Table 2: Effect of different concentration levels of *C. spinosa root* aqueous extract on germination percentage and some growth parameters of *R. sativus* L.

Conc.	GP%	PT%	RL (cm)	PT%	PL (cm)	PT%	SD wt. (mg/seedlings)	SVI
Control	100	0	5.15 ±1.71	0	6.3 ±1.52	0	0.103 ±0.01	1145
5%	97.5	2.50	6.4 ±3.78	0	7.44 ±1.52	0	0.122 ±0.01	1349.4
10%	98.75	1.25	3.59 ±0.92	30.29	8.18 ±2.10	0	0.81 ±0.22	1162.2
20%	100	0	35 ±2.882.	68.74	8.78 ±2.39	0	0.09 ±0.05	1466
40%	25	75	0.33 ±0.04	95.25	0	100	0.11 ±0.01	8.25
5% L.S.D	-	-	1.25	-	2.34	-	0.69	-

GP: germination percentage, PT: Phytotoxic, SVI: Seedling Vigor Index, PL: Plumula length, RL: Radical leng

Table 3: Effect of different concentration levels of *C. spinosa* fruit aqueous extract on germination percentage and some growth parameters of *R. sativus* L.

Conc.	GP%	PT%	RL (cm)	PT%	PL (cm)	PT%	SD wt. (mg/seedlings)	SVI
Control	100	0	7.97 ±2.99	0	8.33 ±1.63	0	0.08 ±0.04	1630
5%	100	0	5.5 ±2.20	30.99	12.04 ±2.57	0	0.11 ±0.14	1754
10%	99	1	5.6 ±3.03	29.73	10.47 ±3.25	0	0.12 ±0.05	1590.9
20%	95	5	2.51 ±2.17	68.50	4.84 ±1.72	41.89	0.11 ±0.08	698.2
40%	91	9	0	100	0.59 ±0.68	92.91	0.13 ±0.09	53.69
5% L.S.D	-	-	1.65	-	2.04	-	3.06	-

GP: germination percentage, PT: Phytotoxic, SVI: Seedling Vigor Index, PL: Plumula length, RL: Radical length

Table 4: Effect of different concentration levels of *C. spinosa leaf* aqueous extract on germination percentage and some growth parameters of *T. aestivum* L.

Conc.	GP%	PT%	RL (cm)	PT%	PL (cm)	PT%	SD wt. (mg/seedlings)	SVI
Control	100	0	10.85 ±1.56	0	9.31 ±0.98	0	0.30 ±0.12	2016
5%	95	5	5.32 ±1.47	50.96	6.68 ±1.26	28.24	0.34 ±0.07	1140
10%	90	10	5.27 ±1.76	51.42	7.6 ±0.97	18.36	0.37 ±0.09	1158.3
20%	76	24	0	100	0	100	0.43 ±0.01	0
40%	50	50	0	100	0	100	0.49 ±0.14	0
L.S.D 5%	-	-	2.65	-	2.97	-	0.99	-

GP: germination percentage, PT: Phytotoxic, SVI: Seedling Vigor Index, PL: Plumula length, RL: Radical length

Table 5: Effect of different concentration levels of *C. spinosa* root aqueous extract on germination percentage and some growth parameters of *T. aestivum* L.

Conc.	GP%	PT%	RL (cm)	PT%	PL (cm)	PT%	SD wt. (mg/seedlings)	SVI
Control	100	0	7.51 ±1.45	0	6.63 ±0.87	0	0.45 ±0.05	1414
5%	100	0	9.45 ±1.88	0	4.6 ±1.73	30.61	0.44 ±0.21	1405
10%	95	5	7.79 ±1.42	0	5.03 ±1.05	24.13	0.33 ±0.14	1217.9
20%	85	15	7.33 ±1.43	2.39	4.4 ±0.69	33.63	0.44 ±0.14	997.05
40%	70	30	1.28	82.95	0.5 4 ±0.41	92.45	0.48 ±0.08	124.6
L.S.D 5%	-	-	2.91	-	1.03	-	1.23	-

GP: germination percentage, PT: Phytotoxic, SVI: Seedling Vigor Index, PL: Plumula length, RL: Radical length

Table 6: Effect of different concentration levels of *C. spinosa* fruit aqueous extract on germination percentage and some growth parameters of *T. aestivum* L.

Conc.	GP%	PT%	RL (cm)	PT%	PL (cm)	PT%	SD wt. (mg/seedlings)	SVI
Control	100	0	7.66 ±1.56	0	6.89 ±1.04	0	0.79 ±0.14	1455
5%	95	5	10.8 ±2.20	0	6.63 ±0.80	3.77	0.47 ±0.07	1655.8
10%	85	15	4.58 ±0.85	40.20	5.29 ±1.07	23.22	0.46 ±0.09	838.9
20%	70	30	1.48 ±1.12	80.67	1.92 ±1.17	72.13	0.57 ±0.09	238
40%	50	50	0.38 ±0.26	95.03	0.66 ±0.51	90.42	0.53 ±0.01	52
L.S.D 5%	-	-	1.35	-	2.06	-	1.33	-

GP: germination percentage, PT: Phytotoxic, SVI: Seedling Vigor Index, PL: Plumula length, RL: Radical length

Antifungal activity

All *C. spinosa* organs extracts had different degrees of antifungal activity against the tested fungi, the highest antifungal activity was recorded for fruit ethanolic extract against *Trichoderma* whereas, acetone extract was the slightest effective extract against the tested fungi. Generally, tested fungi *Trichoderma* and *Aspergillus* were found to be more sensitive to *C. spinosa* organs extracts while *Penicillium* showed high resistance (table 7). The antifungal activities of ethanolic extract of (*Capparis spinosa* L.) was investigated *in vitro* against *Alternaria alternata, Fusarium oxysporum, Phoma destructiva, Rhizoctonia solani,* and *Sclerotium rolfsii* at concentrations of 0, 3, 6, and 9% (v/v) and produced concentration dependent fungal growth inhibition [10]. The antifungal activities of *C. spinosa* organs extracts are attributed to chemical compounds, belonging to secondary metabolites groups such as coumarins, steroids, phenolics , flavonoids, isoflavonoids, alkaloids and other compounds, the richness of the *C. spinosa* with the total phenolic compounds, rutin, tocopherols, carotenoids, and vitamin C could be the main factor in its antifungal effects [19]. Previous chemical studies have reported that alkaloids, lipids, polyphenols, flavonoids, indole, and glucosinolates were isolated from caper extract [20].

Table 7. Antifungal activity of *C. spinosa* extracts against plant pathogenic fungi.

Donor species organs extracts	Organic solvents	Growth-inhibition zone (GIZ) / mm of plant pathogenic fungi		
		Aspergillus niger	*Penicillium sp*	*Trichoderma viride*
Leaves	Methanol	0.3	0.3	0.5
	Ethanol	0.1	0.0	0.0
	Acetone	0.0	0.0	0.0
Fruits	Methanol	0.8	0.0	0.0
	Ethanol	0.7	0.0	0.9
	Acetone	0.3	0.0	0.0
Roots	Methanol	0.5	0.0	0.0
	Ethanol	0.3	0.4	0.0
	Acetone	0.4	0.0	0.0

CONCLUSION

This study demonstrated that the ability of *C. spinosa* organs extracts could be used as environmentally safe alternatives to herbicides for the weed control. In addition, can be used as a biopesticide as an alternative management methods against plant diseases, and control the growth of plant pathogenic fungi and may be applied as an alternative method to reduce fungicide and we recommended to conduct the *in vitro* test followed by the field test. The use of plant extract alone may give no satisfied result, but when it is combined with other measure may give better control level against fungal diseases. Isolation and identification of active compounds in the plant extract that responsible for antifungal activity is needed, in order to assess the efficacy, mode of action and possible side effects of their use.

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