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RESEARCH ARTICLE

In Vitro Antifungal Activity of Different Plant Hormones on the Growth and Toxicity of *Nigrospora* Spp. on Date Palm (*Phoenix dactylifera* L.)

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Abstract:

Objective:

The profound negative impact of chemical fungicides on human and animal health, as well as, the whole agroecosystem encouraged tremendous efforts to find alternative approaches to suppress the growth of plant pathogens.

Method:

Recently, plant hormones have been considered to reduce fungal severity. Five different plant hormones namely 2, 4-D (2,4-Dichlorophenoxyacetic acid); BAP (6- Benzylaminopurine); Dicamba (3,6- Dichloro-2-methoxybenzoic acid, 3,6-Dichloro-o-anisic acid); IAA (Indole-2-acetic acid) and SA (Salicylic acid) were selected to examine their antifungal activity against the growth of two species of date palm fungal pathogen *Nigrospora* spp.

Results:

Results showed that SA at 50 ppm was sufficient to inhibit the mycelium growth of *N. oryzae* completely, while with *N. sphaerica*; the treatments of 2, 4-D (40-50 ppm) and SA (40-50 ppm) led to similar complete inhibition results of mycelium growth. The data of BAP and IAA indicated no toxic effect toward mycelium growth of the pathogens. Similar trends of results have been obtained for phytotoxicity bioassay which performed on detached date palm leaves, 2, 4-D and SA at 30 ppm led to a complete inhibition for the production of toxins in the culture of *N. oryzae* and *N. sphaerica*.

Conclusion:

58% significant reduction in toxin production was obtained with Dicamba 30 ppm. Results presented here reveal the antifungal activity of different plant hormones in *in vitro* experiments, and are important to examine their efficiency in farther field studies on date palm.

Keywords: Antifungal, Date palm, Fungi, *Nigrospora*, Phytohormones, Phytotoxicity.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious monocotyledon of the family Arecaceae, this species is grown mainly in the Middle East and in other family regions around the world [1]. The total production of date palm is approximately 7.4 million tons, and most of this amount comes from the Middle East. Date palm has a pivotal role as ornamental plants, as well as, for their nutritive fruits [2]. Date fruits are a well-known source for energy; and are composed of 70% carbohydrates, mostly sugars, and 15–30% water. Additionally, dates are a good source of different minerals, including iron, potassium, and calcium, as well as low amounts of sodium and fat [3].

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Plants are constantly exposed to different types of stresses during their life cycle, including biotic stresses such as pathogens, weeds, mites, nematodes, and insects, as well as abiotic ones including drought, salinity, pollution, UV light, heavy metals and high or low temperature [4 - 6].

The responses of plants to biotic and abiotic stresses are complex and include several physiological, molecular and cellular modifications; after the perception and recognition of stress by plant cells, the activation of specific ion channels and kinase cascades occurred, the accumulation of reactive oxygen species and plant hormones also followed [7 - 9]. The reprogramming of molecular responses (by altering the expression of different genes involved various pathways) consequently stimulated in plant cells in order to restrict the damage caused by a specific stress [10, 11].

Plant hormones (phytohormones) are naturally occurring organic substances which at low concentration influence the plant life. The most important plant hormones are auxins, gibberellins, cytokinins, ethylene, salicylic acid, jasmonic acid and abscisic acid [12]. Plant hormones are known to regulate the protective responses of plants to both biotic and abiotic stresses, and numerous aspects of plant growth and development [13]. Additionally, plant hormone signals were investigated as individual pathways in mediation of a specific response to a stress. Plant hormones are identified as functioning in complex signalling pathways, by means of synergistic or antagonistic interactions referred as a signalling crosstalk [14].

Furthermore, plant hormones are involved in plant pathogens susceptibility, and affect pathogens behaviour before or after plant attack, several studies have investigated the role of different plant hormones in plant- pathogen interaction, The results of Elad [15 - 17] showed the inhibition activity of auxin indole acetic acid (IAA) at different concentrations on the pathogenecity of *Botrytis cinerea* on aubergine; a significant reduction in disease incidence has been reported. More interestingly; at low concentrations (10^{-4}) IAA led to a significant reduction in *B. cinerea* spore germination *in vitro* or *in vivo*.

Both 2, 4-D (2, 4- dichlorophenoxy acetic acid) and IAA affect the growth (mycelial growth and sporulation) of grey mould fungal pathogen [17]. In terms of cytokinins and their role in fungal severity, there are only few studies related to this subject, the growth of *B. cinerea; B. allii* and *Colletotrichum dematium* as mycelial growth and sporluations were found to be inhibited at increased concentrations of different cytokinins including 6-benzyladenine and kinetin [17, 18].

The *in vitro* study of Alam *et al.* [19] showed that the mycelial growth of anthracnose fungal pathogens (*C. coccodes; C. dematium* and *C. gleosporioides*) was inhibited at the treatments of benzyladenine purine (BAP) and IAA.

Salicylic acid (or ortho-hydroxy benzoic acid) is a natural phenolic compound present at different levels in a wide range of plant species [20]. The increase in SA levels as a response to pathogen attacks was observed in different plants such as tobacco, Arabidopsis and cucumber, this increase was accompanied by systemic acquired resistance (SAR) to fungal pathogen attacks [21, 22]. The antifungal properties of SA have been studied ; Qin *et al.* [23] have reported that SA at concentration of 100 mM was toxic to the spore germination and germ tube elongation of two fungal pathogens, *Penicillium expansum* and *Alternaria alternata*. Similar antifungal activity was revealed with different plant pathogen including *Fusarium oxysporum* f.sp. *niveum* [24]. Many other studies revealed the significant reduction in the *in vitro* growth of *Magnoporthe oryzae*; *Candida* spp.; *Pythium* spp. and *Fusarium* sp [8, 25 - 27].

Two different species of the fungal genus *Nigrospora oryzae* and *N. sphaerica* have been isolated and identified as true endophytic pathogens on date palm leaves (*Phoenix dactylifera* L.) with severe symptoms on young date palm trees [28 - 30].

There is no previous work regarding *in vitro* antifungal activity of different plant hormones on the growth of *Nigrospora* species; therefore, the present study has been conducted.

MATERIALS AND METHODS

Fungal Pathogen Isolates

N. oryzae and *N. sphaerica* were isolated previously from heavily infected date palm leaves with spot symptoms (Figs. **1** and **2**), and identified according to their microscopic features and ITS sequences [30]. Each species were cultured on PDA plates and maintained at 25±2 º C.

Plant Hormones

All examined plant hormones were ordered from Sigma-Aldrich, with the following chemical features:

Plant Hormone	Name	Molecular Weight (g/ mol)
2, 4- D	2,4-Dichlorophenoxyacetic acid	221.04
BAP	6- Benzylaminopurine	225.26
Dicamba	3,6- Dichloro-2-methoxybenzoic acid, 3,6-Dichloro-*o*-anisic acid	221.04
IAA	Indole-2-acetic acid	175.18
SA	2-Hydroxybenzoic acid OR Salicylic acid	138.12

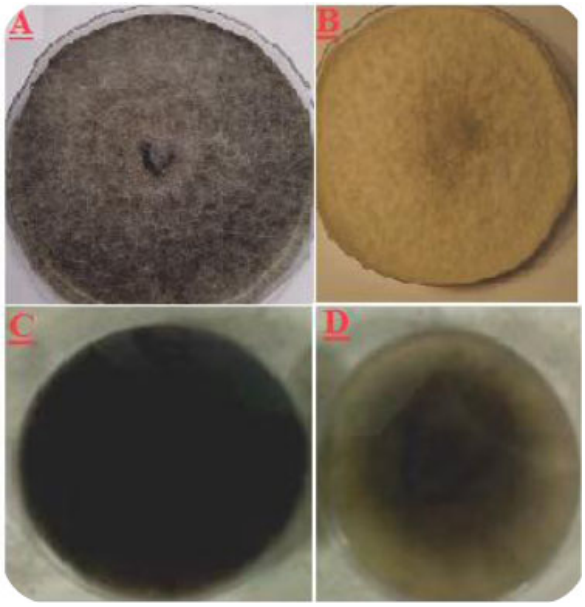

Fig. (1). 7 days growing culture of **A.** *N. oryzae* and **B.** *N. sphaerica* on PDA plate. Reverse growth of **C.** *N. oryzae* and **D.** *N. sphaerica* on PDA plate.

Fig. (2). Fungal leaf and stem spot symptoms. **A.** *N. oryzae* and **B.** *N. sphaerica*.

The Antifungal Activity of Different Plant Hormones on *N. oryzae* and *N. sphaerica* Mycelial Growth

Six concentrations of each plant hormones were tested for their antifungal activity using PDA medium, the concentrations were 0, 10, 20, 30, 40 and 50 ppm. The procedures of Alam *et al.* [19] and Bartoncelli *et al.* [27] were followed. Briefly, after preparation of PDA medium, the phytohormones were added to obtain final concentrations; all media were adjusted to pH of 6.5 by using 0.1 N HCl and NaOH and autoclaved [19], with an exception for Dicamba which sterilised by filter sterilization and added after autoclaving. 20 millilitres of each treated medium was poured into Petri dish of 9 cm. The inoculation of the centre of PDA+ hormones medium was followed using a 0.5 cm of newly mycelium growth of each examined fungi and incubated at 25±2 ºC, the measurement of radial growth of mycelium was taken when the growth of each fungus reached the edge of control treatment (0 concentration), by measuring the diameter of each colony in two directions at right angles to each other [29].

The Inhibitory Activity of Different Plant Hormones on the Phytotoxicity of *N. oryzae* and *N. sphaerica* Cultural Filtrates

Different concentrations of plant hormones (2, 4-D 30; BAP 50; Dicamba 50; IAA 50 and SA 30 ppm) were selected to test their inhibition activity on the production of plant toxins in fungal cultural filtrates. Each fungus (*N. oryzae* and *N. sphaerica*) were grown in the following toxin production medium: 30 g glucose, 3 g $NaNO_3$, 1g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4.7H_2O$, 1 g yeast extract and water up to 1 L [28]; in addition to plant hormones. The cultures were kept at 25 º C on a rotary shaker (180 rpm for 10 days according to [29]) and the culture filtrate was then collected and freeze-dried. The procedure of Fukushima *et al.* [31] was followed with some modification. Briefly, the crude filtrate was dissolved in 70% acetone and 0.1% Tween 80. Puncture wounds were performed on date palm cultivar of 'Al-Sayer' leaves, and a 5 µL droplet of the crude extract were placed on each wound, the leaves were kept at 25 ºC for 7 days and the diameters of necrotic lesion were measured. A control treatments were done by application of a solution of 70% of acetone and 0.1% of Tween 80 only as a negative control, while the positive ones were performed using each examined cultural filtrates of *Nigrospora* species and considered in statistical analysis.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare experiment results according to randomized design. The mean differences were compared by revised least significant difference (RLSD) test at $P \leq 0.01$ levels. The obtained data were analyzed statistically with SPSS-21 statistical software (SPSS In., Chicago, IL., USA). The bars on each diagram represent standard deviations. Each data of tables and figures represent the means of five replicates, and experiments were repeated twice.

RESULTS

The Antifungal Activity of Different Plant Hormones on *N. oryzae and N. sphaerica* Mycelial Growth

Results of antifungal activity on PDA plates, showed that the responses of *N. oryzae* and *N. sphaerica* to plant hormones were varied according to the type of plant hormones and their examined concentrations; in regards to SA treatment at 50 ppm, results showed a complete inhibition of *N. oryzae* mycelium growth, similar inhibition was observed with *N. sphaerica* at the treatments of 2, 4-D (40-50 ppm) and SA (40-50 ppm) (Table **1**).

The data for BAP (10-30 ppm) and IAA (10-30 ppm) indicated no toxic effect of these chemicals toward the mycelium growth of the pathogen *N. oryzae*; with no significant differences than control treatment (no hormone treatment). A range of concentrations, 10-40 ppm for BAP and IAA have no inhibitory effect toward the growth of *N. sphaerica*.

Overall results, showed that the sensitivity of *N. sphaerica* mycelium to all examined plant hormones was greater than those observed in the mycelium of *N. oryzae*, e.g., at the treatment of 2,4-D (20 ppm), the growth was 7.60 cm (inhibition percent of 15.50%) in *N. oryzae*, while it was 3.70 cm (inhibition percent of 58.50%) in *N. sphaerica*. The average of mycelium growth was 5.59 cm in the species *N. sphaerica*, compared with was observed at the fungus *N. oryzae* (6.17 cm).

Regarding the type of plant hormones and their effect on fungal growth, statistical analysis revealed that the treatment of SA reported the highest inhibition percentage which was 70.34% and an average of growth reached 2.67 cm compared to control one. Followed by 2,4-D and Dicamba treatments (4.07 and5.29 cm; respectively). No inhibition

effect was seen with the treatment of IAA compared to control one.

Additionally, our results revealed that the inhibition of fungal growth increased with the increasing of chemical concentrations, in 10 ppm treatment of 2,4-D the mycelium growth was 8.10 cm, this growth was significantly reduced up to 4.5 folds (2.62 cm) at 50 ppm for *N. oryzae* mycelium growth.

Table 1. The effect of different plant hormones (ppm) on the mycelial growth of *N. oryzae* and *N. sphaerica* on PDA plates. Values represent the average of five replicates for each treatment ± standard deviation value.

Plant Hormones	Concentration (ppm)	Fungal Mycelial Growth (cm)		Plant Hormones Average
		N, oryzae	*N, sphaerica*	
2, 4-D	0	9.00±0.00	9.00±0.00	**4.07**
	10	8.10±0.20	6.90±0.17	
	20	7.60±0.38	3.70±0.50	
	30	5.30±0.36	1.60±0.32	
	40	4.90±0.20	0.00±0.00	
	50	2.60±0.50	0.00±0.00	
BAP	0	9.00±0.00	9.00±0.00	**8.46**
	10	9.00±0.00	9.00±0.00	
	20	9.00±0.00	9.00±0.00	
	30	8.40±0.30	9.00±0.00	
	40	7.80±0.20	8.10±0.30	
	50	7.40±0.25	7.80±0.35	
Dicamba	0	9.00±0.00	9.00±0.00	**5.29**
	10	8.20±0.25	7.90±0.30	
	20	7.20±0.30	4.90±0.52	
	30	5.90±0.30	3.90±0.20	
	40	5.00±0.26	3.60±0.49	
	50	4.40±0.20	1.90±0.30	
IAA	0	9.00±0.00	9.00±0.00	**8.90**
	10	9.00±0.00	9.00±0.00	
	20	9.00±0.00	9.00±0.00	
	30	9.00±0.00	9.00±0.00	
	40	8.80±0.10	9.00±0.00	
	50	8.60±0.15	8.67±0.15	
SA	0	9.00±0.00	9.00±0.00	**2.67**
	10	7.80±0.40	5.20±0.30	
	20	5.80±0.025	2.96±0.20	
	30	3.40±0.47	0.97±0.15	
	40	1.20±0.15	0.00±0.00	
	50	0.00±0.00	0.00±0.00	
Average of Fungal Species		**6.17**	**5.59**	

$RLSD_{(0.01)}$: For N. oryzae and hormones = 0.49, For N. sphaerica and hormones = 0.39, For fungal species = 0.08, For plant hormone = 0.132.

The Inhibitory Activity of Different Plant Hormones on the Phytotoxicity of *N. oryzae* and *N. sphaerica* Cultural Filtrates

The full crude extracts of *N. oryzae* and *N. sphaerica* (grown on liquid medium supplemented with plant hormones) were introduced into detached leaves of date palm "Al-Sayer" as a sensitive cultivar [30] according to the procedure of Fukushima *et al.* [31] to detect the effect of different plant hormones on the phytotoxicity of examined fungi. Results showed that at control treatment (no hormone), the first necrotic lesions were appeared after 3 days of treatment (5 µl of the crude extract) with *N. oryzae* and *N. sphaerica*; the lesion diameters were 1.33 and 1.03 cm; respectively. These lesions were extended rapidly at 6 and 9 days post-inoculation, with evident phytotoxic effect at 9 days, the increase level was up to 2.5 folds, compared with 3 days after treatment (Figs. **3A** and **D**).

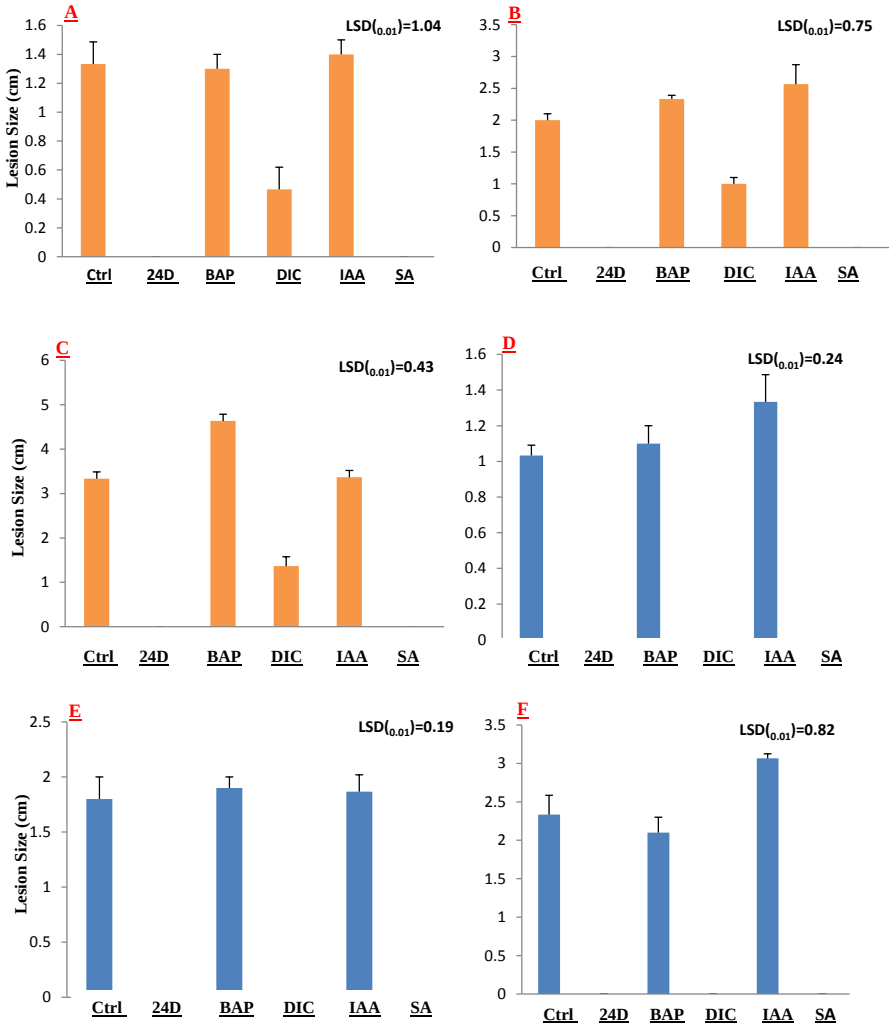

Fig. (3). The effect of different plant hormones on the phytotoxicity of *Nigropsora* spp. cultural filtrates on date palm "Al-Sayer" detached leaves.

Values represent the average of five replicates for each treatment ± standard deviation value. Different letters indicate significant differences between treatments according to LSD test at 0.01.

A: 3 days post-inoculation for *N. oryzae*. **D:** 3 days post-inoculation for *N. sphaerica*.

B: 6 days post-inoculation for *N. oryzae*. **E:** 6 days post-inoculation for *N. sphaerica*.

C: 9 days post-inoculation for *N. oryzae*. **F:** 9 days post-inoculation for *N. sphaerica*.

Ctrl: Positive control treatment; **24D**: 2,4-D at 30 ppm treatment; **BAP**: BAP at 50 ppm; **DIC**: Dicamba at 50 ppm; **IAA**: Indole acetic acid at 50 ppm; **SA**: Salicylic acid at 30 ppm.

Statistical analysis revealed that the treatments of BAP and IAA at 30 ppm had no significant effect on the phytotoxicity of both *N. oryzae* and *N. sphaerica* at all periods of study (Figs. **3A** and **F**), while Dicamba at 30 ppm led to a significant reduction in toxin production of *N. oryzae* up to 58%, the lesion diameter was 3.32 in *N. oryzae* control treatment and reached 1.37 cm in *N. oryzae* + Dicamba (Fig. **3C**). Its noteworthy, that the concentration of 30 ppm of 2, 4-D and SA was sufficient to inhibit the production of toxins completely in the culture of *N. oryzae* and *N. sphaerica* (Figs. **3F** and **C**).

DISCUSSION

The genus *Nigrospora* has been well studied as a plant endophytic pathogen, with a considerable attention for two species *N. oryzae* (Berk and Broome) Petch and *N. sphaerica* (Sacc.) Mason. Abass *et al.* [28 - 30] isolated and identified these two species from heavily infected date palm leaves with leaf spot disease. In addition to date palm,

several other important plants have been reported as a host for *N. oryzae* including rice and maize disease [32, 33]. Recently, a growing number of researches proved the ability of *N. oryzae* to invade different plants such as kentucky bluegrass (*Poa pratensis* L.); sesame (*Sesamum indicum* L.) and wheat (*Triticum aestivum* L.) [34 - 36]. In regards to the species of *N. sphaerica* several studies proved their ability to induce different disease symptoms on many plant hosts such as banana and blueberry plants (*Vaccinium corymbosum* L.) [37, 38]. More recently, on tea (*Camellia sinensis* L.); calabash [*Lagenaria siceraria* (Molina) Standl.] and Pitaya (*Hylocereus undatus* Britt) [39 - 41].

The obtained results proved that 2,4-D and SA at 50 ppm were able to inhibit the mycelium growth of *N. oryzae* and *N. sphaerica* in amended PDA plates completely, the significant reduction in pathogens growth is in a good agreement with many other *in vitro* studies revealed the inhibition efficiency of 2,4-D toward various plant pathogens, at concentration of 50 ppm a significant inhibition was reported in the growth of anthracnose pathogens (*C. coccodes; C. dematium* and *C. gleosporioides*) [19], in addition, 2,4-D was found to be a potential *in vitro* suppressor for the spore germination and growth of the fungus *Harpophora maydis* the cause of late wilt in maize [42].

The present findings of SA antifungal activity against leaf spot pathogens were in accordance with those of Saikia *et al.* [43] and Abdel-Moniam [44] who reported that the *in vitro* growth of fungal pathogens *F. oxysporum* f. sp. *ciceri* and *F. oxysporum* f. sp. *lycopersici* (FOL) completely stopped in PDA plates amended with SA. An observation of macroconidium germination inhibition was also revealed after treatment of *F. graminearum* cultures with salicylic acid [45]. Similar effect was reported in the study of Sedghi and Gholil-Toluie [26] where SA treatments led to a good inhibitory for mycelium growth of various plant pathogens including *Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp., *Phomopsis* spp. and many other pathogens. Also, SA at a concentrations of 10-25 ppm were observed to inhibit the mycelium growth of *Fusarium* spp., *B. cinerea*, *P. aphanidermatum*, *R. solani* and *Alternaria solani* completely, in PDA- plate assay [46].

Our results showed that the inhibitory effect of SA and 2,4-D were varied according to the examined concentrations, and this is in accordance with results of the results of Alam *et al.* [19] on concentrations dependent manner of 2,4-D toward a wide range of anthracnose pathogens, as well as, Abdel-Monaim, *et al.* [44] when they showed that the fungitoxicity of SA against FOL was dependent on concentration tested.

Many explanations have been postulated to clarify the *in vitro* fungitoxicity of these hormones including: (1) suppression effect on the spore formation and germination [24, 44] (2) damaging on lipid level; leakage of pathogen's proteins and intracellular disorganization [47] (3) affecting hyphal branching patterns and delaying in spore germination [48].

In regards to IAA and BAP results, a weak inhibition response was reported in both *N. oryzae* and *N. sphaerica* pathogens, which were less than 18 and 4% as a percentage of mycelial growth inhibition, respectively. This weak inhibition response is in agreement with the results of Alam *et al.* [19] who reported that both IAA and BAP led to a weak inhibition (4 and 11%; respectively) to the mycelium growth of anthracnose fungal pathogen (*Colletotrichum* spp.). Petti *et al.* [49] reported that IAA has no effect on *F. culmorum* growth when using a nutrient-rich medium.

Interestingly, an enhancement of sporulation and spore germination was found as a consequence of IAA and BAP treatment with *B. cinerea* and *B. allii* pathogens [50, 51]. In terms of Dicamba antifungal activity, our results showed a good level of inhibitory effect toward *N. oryzae* and *N. sphaerica* which were 51 and 78.80%, as mycelium growth inhibition percent, however, to the best of our knowledge, this is the first demonstration of Dicamba repressive activity on fungal growth.

It is noteworthy that the sensitivity of *N. sphaerica* was greater than those observed with *N. oryzae* to all examined phytohormones and concentrations, In previous study, the results of susceptibility test of different date palm cultivars revealed higher levels of virulence of *N. oryzae* compared to *N. sphaerica*, in addition to high enzymatic activity (cellulase; lipase and protease) [30]; thus, could be provide an explanation for fungal responses to plant hormones.

Regarding the phytotoxic effect of *Nigrospora* spp. cultural filtrates on date palm detached leaves using leaf puncturing assay, results showed that the first necrotic lesion was induced after 3 days post inoculation for two examined species. Diameters of and 1.30 and 1.03 cm were reported in the treatment of *N. oryzae* and *N. sphaerica*, respectively.

The phytotoxic effect of *Nigrospora* cultural filtrates could be attributed to the toxins secreted by these pathogens, different toxins have been isolated and identified in the crude cultures such as lactones, the results of Harwooda *et al.* [52], revealed that the lactone named nigrosporolide was isolated from the culture of *N. sphaerica* and found to be a

strong inhibitor for the growth of wheat coleoptiles. Additionally, Fukushima *et al.* [31] showed that purified phomalactone was able to induce water-soaked necrosis on leaves after 24 h of injection at 1000 ppm.

In addition to lactones; several phytotoxins have been purified and characterised according to their phytotoxicity, including aphidicolin; aphidicolene; aphidicolaneodiol; aphidicolanatriol; and aphidicolanepentol from the culture of *N. oryzae*, with a strong inhibition activity toward DNA polymerase [53].

The results herein are in accordance with the results of Tanaka *et al.* [54] and Abass *et al.* [29] when they used leaf puncture assay to reveal the phytotoxicity of *N. oryzae* and *N. sphaerica* on different plants including green foxtail; barnyard grass; velvet leaf; corn; cowpea and date palm. The phytotoxicity of *Nigrospora* spp. could be explained by the inhibition activity against (1) starch synthesis (revealed in barnyard grass plant) (2) photosynthetic CO_2 fixation (revealed in *Chlorella vulgaris* algae) (3) root elongation (revealed in lettuce plant) (4) oxygen evolution (revealed in pumpkin plant) (5) inducing of electrolyte leakage from photosynthetic tissues of cucumber cotyledons and *Zinnia elegans* leaves [54, 55].

In regards to the interaction between phytohormones and phytotoxicity of cultural filtrates of *Nigrospora* spp., results showed a slight increase of the phytotoxicity of both examined species in IAA and BAP at 30 ppm during inoculation periods, these findings are in a good agreement with the results of Farag *et al.* [56] when they showed that the addition of indole acetic acid to the medium increased aflatoxin B production of the fungus *Aspergillus parasiticus*; also, Luo *et al.* [48] showed a small positive effect of IAA on mycotoxin 15-ADON production by *F. graminearum*. Opposite trend of results was found with 2, 4-D and SA, which led to a complete inhibition in toxin yields of two fungal pathogens in liquid medium, such inhibition has been reported in the study of Panahirad *et al.* [57] when they applied SA on pistachio fruits to control the *A. flavus* infection and reduce aflatoxin production. To the our best of knowledge, this is the first study on the interaction between 2,4-D and Dicamba with fungal mycotoxin production.

CONCLUSION

In this study, it was proved that the responses of *Nigrospora* species to plant hormones were varied according to the type and concentration of hormones used. A potential antifungal activity was observed at the treatments of 2,4-D; dicamba and SA on the *in vitro* growth of *N. oryzae* and *N. sphaerica*, especially at concentrations of 30-50 ppm. In addition to inhibition of *Nigrospora* growth, 2,4-D; dicamba and SA led to a significant inhibition of mycotoxins production in liquid medium which approved by leaf puncture assay on date palm detached leaves. More interestingly, both IAA and BAP hormones induced a slight increase in the phytotoxicity of examined fungi. Further field studies are required to investigate the role of these hormones as fungicides in control of *Nigrospora* spp. on date palm.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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Declared none.

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