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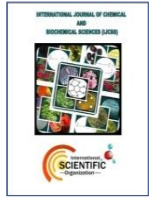

Sesame phyllody incidence – vector insect's relationship, biochemical response and phytoplasma DNA detection in infected plants

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Abstract

The present studies were carried out in farmer fields cultivated with sesame variety Shandaweel *Sesamum indicum* L at Abo Hamad, Sharkia Governorate, Egypt, during the two seasons of 2021 and 2022 to study Sesame phyllody incidence – vector insect's relationship, biochemical responses and phytoplasma DNA detection in infected plants. The obtained results cleared highly significant differences between the phyllody disease incidence mean at different inspection times ($P<0.001$) during the two seasons. The seasonal accumulated infection percentages were ranged between 24.96 to 25.68 % during 2021 and 2022 seasons, respectively. The infection rates were increased from flowering start time to the harvesting time. The infection mean rats of the two study seasons were differed insignificantly ($p>0.05$), while the grand mean of infection percentages at different inspection times (ranged between 1.075 to 18.27 %) were varied with highly significant differences in accordance to inspection times ($P<0.001$). Also, the results cleared that, insignificant correlation was found between the total numbers of main Piercing-sucking insect's species (leafhoppers, Green peach aphids and Green stink bug) and phyllody incidence. The physiological responses of sesame plants to phytoplasma infection were estimated as the content of total soluble protein, lipids, carbohydrates, and phenol oxidase in infected plants and compared with healthy ones. The obtained results indicated that the phytoplasma infection reduced total soluble protein with 90.031 and 59.725%; Phenol oxidase by 48.569, 84.435 % in leaves and buds of infested sesame plants, respectively. In the contrast, the total lipids were increased in infected sesame leaves only and total carbohydrate increased in infected leaves and buds. These results cleared that the infection affected plant resistance by reducing phenol oxidase compound and guide plants to vegetative growth by increasing the carbohydrates and lipids in sesame plants. As biotechnological studies to Detect phytoplasmas DNA, RNA in sesame plants the Ethidium bromide-stained gel of PCR amplification products obtained by using universal primer pair P1/P7 in PCR amplification for phytoplasma detection. Also, Nested primer R16F2n/R16R2 was used and confirm the infection with a product size range about 1200 bp for infected sample, while no results were shown in healthy plants.

Keywords: Sesame phyllody Incidence, biochemical responses and phytoplasma DNA detection.

Full length article **Corresponding Author*, e-mail: hamzash@hotmail.com

1. Introduction

Sesame, *Sesamum indicum* L. (Fam: Pedaliaceae) is one of the ancient oilseeds crops. The sesame oil is easiness of extraction, great stability, and resistance to drought. Phytoplasma (similar to mycoplasma obligate parasites) is one of the most dangerous pathogens that affect sesame and other plants world-wide. Phytoplasma cause phyllody character of symptoms such as: stunting, changing the color of leaves from green to yellow and alteration of the floral parts into leafy structures bearing no capsule and seeds. Phytoplasma consider a serious threat disease for the production of sesame in many countries. On the other hand, the phytoplasma effect enzymes in infected plant, such as Peroxidase and Polyphenol oxidase and hormones like Auxins, Cytokinin, Gibberellins and Abscisic acid [1]. Recently, the symptoms of sesame phyllody were observed

to spread in sesame fields**,** many other field and vegetables crop fields in the eastern Delta region. As global literature the symptoms in sesame and other plants ; flower greening (virescence) retrograde metamorphosis of floral organs into leaf structure, reduced leaf size, shortening of internodes and loss of apical dominances, proliferation of shoots, whishes'-brooming (crowding of leaves and small shoots in the apical region); pods cracked, general decline; lead to high yield reduction; [2,3,45,6,7]; they added that the sucking- piercing pests are the main vectors of the pathogen microorganisms like phytoplasma, candidatus phytoplasma strains. Also, the scientists stated that the leafhopper (Empoasca decipiens) was among insect vector of the pathogen [8,9]. In host plants tissues, phytoplasma are restricted to the phloem sieve tubes and are transmitted between plants by phloem –sap-feeding pests in a persistent manner [9]. Phytoplasmas (Formerly mycoplasma like organism, MLO) have diverged from gram-positive bacteria, and belong to the condidatus phytoplasma genus within the class Mollicutus [10,11,12,13].

The study aimed to:
1. Determine phytoplasmas disease incidence in sesame fields.
2. Relationship between the pest vectors occurrence and disease incidence.
3. Test Biochemical response of sesame plants to the phytoplasmas infection.
4. Detection of phytoplasmas DNA and RNA in sesame symptomatic plants.

2. Material and Methods

The present studies were carried out in farmer fields cultivated with sesame variety Shandaweel at 15 and 21 May at Abo Hamad, Sharkia Governorate, Egypt, during 2021 and 2022; seasons to study the incidence of phytoplasmas disease in sesame fields; Test biochemical response of sesame plants to the phytoplasmas infection and detection of phytoplasmas DNA and RNA in sesame symptomatic plants

2.1: Incidence of Phytoplasmas disease in sesame fields

Randomly samples of 25 plant /field were inspected actually in the field depending on the previous visual symptoms found in specific lectures of phytoplasmas. The samples were replicated four times and investigated actually in the fields at three times throughout the growing seasons of sesame plants i.e., flowering start, mid-season and at the end economic harvesting. The phytoplasmas diseases as visual symptoms (symptomatic plants) incidence % means were recorded, tabulated and subjected to statistical analysis for the two study seasons.

2.2: Relationship between the pest vectors occurrence and disease incidence

Survey and population density of Phytoplasmas vectors insects: two sampling techniques were used for surveying and counting Phytoplasmas piercing-sucking vectors insects on sesame plants, i.e.; plant sample (early in the seasons, seedling transferred to laboratory and examined using binocular stereo microscope and when the true leaves formed the plants inspected actually in the field for the last periods) and sweeping net.

2.2.1: Plant samples technique

Weekly samples of 25 seedlings/ replicate (four replicates) were chosen randomly just after emergence of seedlings tell true leaves formation; then 25 leaves / replicate (four replicates) from the different levels of plant canopy were considered for each sample until the end of growing season of sesame plants. Samples were taken early in the morning and kept in tightly closed paper bags and transferred to the laboratory for examination using binocular stereo-microscope (at early season). The all stages individuals of piercing-sucking vectors insects were sorted; identified (for survey) and the numbers of the main species were recorded for samples. Samples of 25 plants /replicate (four replicates) were inspected actually in the field to count all stages of green stink bug was recorded early in the morning.

2.2.2: Sweeping technique

Sweeping net was used to collect flying insects. Samples of 25 double strokes of sweeping net were taken weakly by walking in diagonals in the four replicates in the experimental sesame fields. The catch of each replicate was put in plastic sacks, transferred to laboratory, and then anesthetized using chloroform, where insects were sorted, classified, counted and recorded. The correlation between total numbers of main piercing-sucking vectors insects, disease incidence and some weather factors (temperature and relative humidity means) were inspected.

2.3: Physiological analysis

2.3.1: Total soluble protein determination

Colorimetric determination of total soluble protein in total homogenate infected and healthy sesame plants were carried out as described by [14]. The principle of this method is based on that protein in the presence of an alkaline cupric sulfate, the protein produces a violet purple color, the intensity of which is proportional to their concentration. Briefly, a volume of 0.2 ml of larval homogenate was added to 5ml of Biuret reagent and incubated for 30 min at 20-25℃. The absorbance of the sample against a blank Biuret reagent was measured at wave length of 546 nm.

2.3.2: Total lipids determination

Total lipids were estimated by the method of [15] using phosphovanillin reagent prepared by dissolving of 0.6 gm. pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water. Then 400 ml conc. phosphoric acid was added. 250μl of sample were added to conc, sulphuric acid (5ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the digest was added to phosphovanillin reagent (6 ml) After 45 min, the developed color was measured at 525 nm against reagent blank, O.D was compared to that of a reference standard and results expressed as mg lipids/ gm. body weight

2.3.4: Total carbohydrate determination

The carbohydrates levels were determined according to methods provided by [16].

2.3.5: Phenol oxidase determination

Polyphenol oxidase activity (PPO). The reaction mixture consisted of 100 μl crude enzyme, 600 μl catechol and 2.3 ml phosphate buffer (0.1 M, pH 6.5). The absorbance at 420 nm was recorded at zero time and after 1 min using spectrophotometer. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per min at 420 nm. The enzyme activity was expressed as unit. mg-1 protein [17].

2.4. Detection of phytoplasmas DNA, RNA in symptomatic host plants

2.4.1: DNA Extraction

DNA was extracted from naturally infected and healthy sesame samples using a modified Dellaporta extraction method [18]. Approximately 0.5g of fresh tissue was used for each extraction. 500 µl of Dellaporta extraction buffer (100 mMTris pH 8.0, 50 mM EDTA, 500 mM sodium chloride and 10 mM β-mercaptoethanol), then vortex for 2 min. 33 microliters of 20% sodium dodecylsulphate (SDS) was added and vortex well, then incubated for 10 min at 65 C. 160 µl of 5 M potassium acetate was added and vortex, followed by centrifugation for 10 min at 10.000 rpm. The supernatant (450) was transferred carefully to new eppendorf tube. An equal volume of (PCI) phenol:chloroform:isoamyl alcohol, 25:24:1 (v:v:v) was added and vortexed for 5 min then centrifuged at 10,000 rpm for 5 min. The aqueous layer was transferred to new eppendorf tube. 0.5 Volume of isopropanol was added, centrifuged for 15 min at 15.000 rpm. 500 µl of 70% ethanol was added to wash the pellet and centrifuged for 5 min at 10,000 rpm. The pellet was exposed to air for 15 min. then resuspended in 50 µl of dH2O and stored at −20 °C.

2.4.2: PCR amplification

Amplification of phytoplasma 16S rRNA gene was achieved with primer pair P1/ P7 [19,20] in the direct reaction. The 0.5 µl of each sample of the first reaction products was used as template for nested PCR with primers R16F2n/R16R2 [21]. PCR was performed with 34 cycles in an automated thermal cycler (C1000TM Bio-Rad. PCR products were observed in an ethidium bromide-stained 1.5% agarose gel using an ultraviolet transilluminator.

2.4.3: Sequences of the oligonucleotide primers used for PCR amplification

P1 16S 5'AAGAGTTTGATCCTGGCTCAGGATT3'
P7 23S 5'CGTCCTTCATCGGCTCTT3'
R16F2: TGACGGGCGGTGTGTACAAACCCCG
R16R2: GAAACGACTGCTAAGACTGG

3. Results and Discussion

The present studies were carried out in farmer sesame fields cultivated with sesame variety Shandaweel at 15 and 21 May at Abo Hamad district, Sharkia Governorate, Egypt, during 2021 and 2022; seasons, respectively; to study the incidence of phytoplasmas disease in sesame fields; to test biochemical response of sesame plants to the phytoplasmas infection and to detection of phytoplasmas DNA and RNA in sesame symptomatic plants.

3.1: Incidence of Phytoplasma diseases in sesame fields

Incidence of phytoplasmas disease was observed in the surveyed fields at Abo Hamad district and the two seasons accumulated disease incidence mean reached 25.32% in investigated sesame fields. The prevalent symptoms of diseases were severe little leaves, internode shortening, witches' broom (Fig.,1a,b,c) compared with healthy plants (Fig.,2a,b,c) , flower virescence, phyllody, proliferation and sterility, cracking of sesame seed capsules (Fig. 3). Germination of seeds in capsules, dwarfing, and yellowing. The infection of phytoplasma disease was inspected as phyllody symptoms incidence at three times throughout sesame plants growing season (at flowering start time, mid flower period and at harvest times) during the two seasons of 2021 and 2022 at Abo Hammad district. The obtained results cleared that there were highly significant differences between the phyllody disease incidence mean at different inspection times ($P<0.001$). The infection percentages were ranged between 0.44 – 20.44 % with seasonal accumulated percentage of 24.96 % during 2021 season; where the infection increased from flowering start time to the harvesting time. In the same trend, the infection during the second season was ranged between 1.71 to 16.09 % with seasonal accumulated percentage of 25.68 %. The infection mean rats of the two study seasons were differed insignificantly ($p>0.05$), while the grand infection percentages at different inspection times ranged between 1.075 to 18.27 % were varied with highly significant differences inspection times ($P<0.001$). Generally, the sesame plants found infected with highest percentages reached to 25.32 % as accumulated general mean percentage of the two investigated seasons compared with the other crops; recorded at the third inspection period (harvesting time) at the 1st season. These results cleared that the sesame plants could be infected early in the season but the symptoms not appear and the higher infection was recorded after the higher activity of insect and other phytoplasma vectors. In the same trend many authors stated that, among the diseases, phyllody (caused by phytoplasmas) is a destructive disease of sesame which causing significant economic losses by altering their floral part into leafy structures with no capsule and therefore no seeds consist [22,23]. In Egypt, [1] collected symptomatic samples including green leaf-like floral organs, virescence, phyllody and proliferation from infected sesame field in Giza Governorate Egypt.

3.2: Relationship between the insect vectors mean numbers and disease incidence

The results in Table (2) reveal that, the sesame plants found infested with 8 piercing–sucking insects species. The main and dominant species were leafhoppers species, green peach aphid and green stink bug found infesting sesame plants in relatively high numbers followed by cotton whitefly and cotton aphids in very low numbers. The results in Table (3) cleared that, insignificant correlation was found between the total numbers of main Piercing-sucking insect's species (leafhoppers, Green peach aphids and Green stink bug) and phyllody incidence. The total numbers of insect's species were started in relatively low numbers of 130.25 insects throughout flowering start period in timing with low phyllody incidence 1.075 % under 28.50 - 28.67 ℃ and 52.47 - 50.53 % RH. The relatively high total numbers of insects 202.525 insect were recorded during the mid-flowering period where the phyllody incidence reaches 5.98 % under 31.54 - 30.71℃ and 57.11 - 52.11% RH. At economic harvesting period the phyllody incidence of 18.27 % and the insect's total numbers of 126.705 Insect were recorded under 29.71 - 29.62℃ and 57.5 - 52.47 % RH. These results indicated that the high insect's numbers during the 2nd period resulted in increasing of phyllody incidence in the end of seasons. These results found agree with those of [24,25] who stated that the sesame plant found infested by leafhopper (vector of phyllody) and other sucking pests.

Fig. 1a: Infected sesame plant

Fig. 2a: Healthy sesame plant

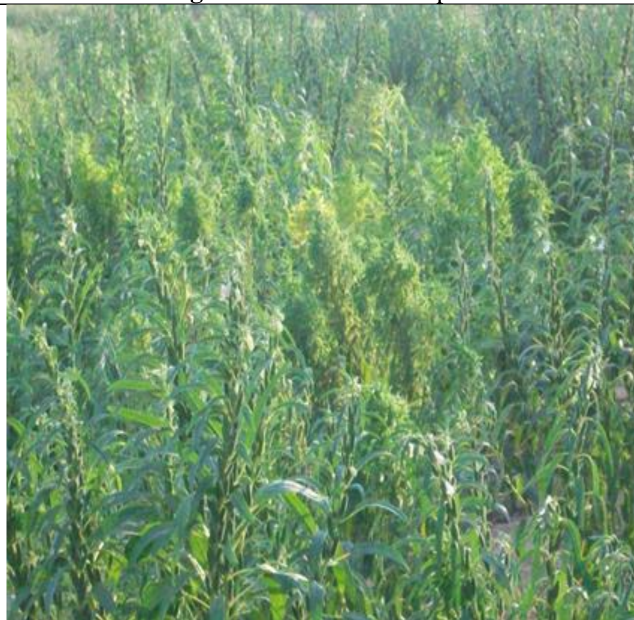

Fig. 1b: Infected sesame field

Fig. 2b: Healthy sesame field

Fig. 1c: Infected sesame plant

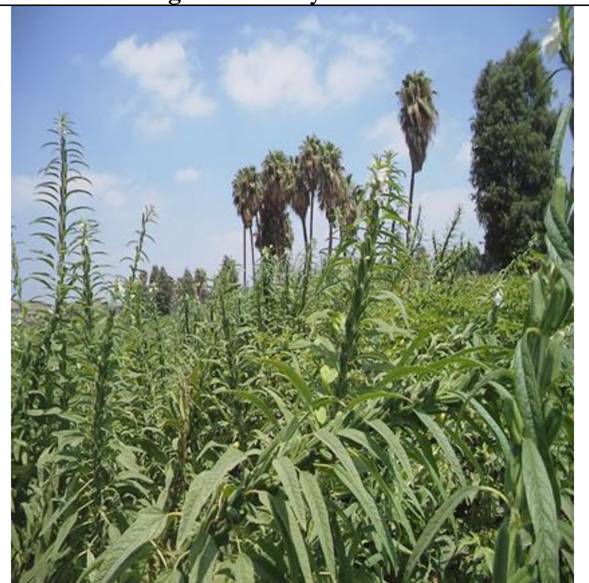

Fig. 2c: Healthy sesame plant

Fig. 3: cracking of sesame seed capsules, germination of seeds in capsules, dwarfing, and yellowing phyllody symptoms

Table 1: Phytoplasma infestation percentages in sesame fields during the two study seasons at Sharkia Governorate, Egypt

Crop	inspect time	season		grand mean
		2021	2022	
sesame	at flowering start	0.44 ± 0.066 c	1.71 ± 0.630 c	1.075± 0.57 c
	at mid flower period	4.08 ±0.814 b	7.88 ± 0.516 b	5.98 ± 1.15 b
	at economic harvesting	20.44 ± 0.402 a	16.09 ± 1.855 a	18.27± 0.58 a
LSD0.05 (probability)		1.819 (P< 0.001)	4.05 (P< 0.001)	2.825 (P< 0.001)

Table 2: Piercing-sucking insect's species associated with Phytoplasmas symptomatic sesame plants at Abo-Hammad district Sharkia, Governorate, Egypt during 2021 and 2022 seasons

English name	Scientific name	Arabic name	Family	Order	Occurrence level
leafhoppers	*Empoasca lybica (de Berg),*	El jassid	Cicadellidae	Hemiptera	+++
	Empoasca decipiens (Poali)				
	Empoasca decedens				
	Orosius SPP.				
Cotton aphids	*Aphis gossypii* (Glov.)	Al men	Aphididae	Hemiptera	+
Green peach aphids	*Myzus persicae* (sulz.)				+++
Cotton whitefly	*Bemisia tabaci* (Genn.)	Zobaba bidaa	Alerodidae	Hemiptera	+
Green stink bug	*Nezara viridula*	Baket khadra	pentatomidae	Hemiptera	+++

Table 3: Total numbers of main piercing-sucking insect's species associated with phytoplasmas symptomatic sesame plants throughout inspection periods at Abo Hammad, Sharkia, Governorate, Egypt during 2021 and 2022 seasons

Inspection periods	**2021**						**2022**					
	Empoasca spp.	M. persicae	N. viridula	total	mean temperature	mean relative humidity	Empoasca spp.	M.persicae	N. viridula	total	mean temperature	mean relative humidity
at flowering start	46.50	36.25	14.75	97.5	28.5	52.47	82.5	77.75	2.75	163	28.67	50.53
at mid flower period	129.50	44.00	9.00	182.5	31.54	57.11	102.50	114.30	6.00	222.80	30.71	52.11
at economic harvesting	65.25	41.00	9.50	115.8	29.71	57.5	49.75	90.16	17.75	157.70	29.62	52.47

Table 4: Biochemical responses of symptomatic host plants to the phytoplasmas infection

Samples	**Total soluble protein**	**Total lipids**	**Total carbohydrate**	**Phenol oxidase**
Sesame healthy leaves	12.97 ± 0.918 a	24.327 ± 0,967 c	175,94 ± 3.55 c	1235.16 ± 7.643 b
Sesame infected leaves	1.293 ± 0,095 c	26.413 ± 0.486 b	180.99 ± 0.759 c	635.26 ± 8.59 c
Infection effect %	-90.031	8.575	2.780	-48.569
Sesame healthy bods	4.37 ± 0.228 b	34.68 ± 0.369 a	211.32 ± 0.543 b	4066.83 ± 13.314 a
Sesame infected bods	1.76 ± 0.211 c	4.33 ± 0.390 d	242.22 ± 1.019 a	633.01 ± 0.86 c
Infection effect %	-59.725	-87.514	14.622	-84.435
Probability	< 0.01	< 0.001	< 0.01	< 0.01

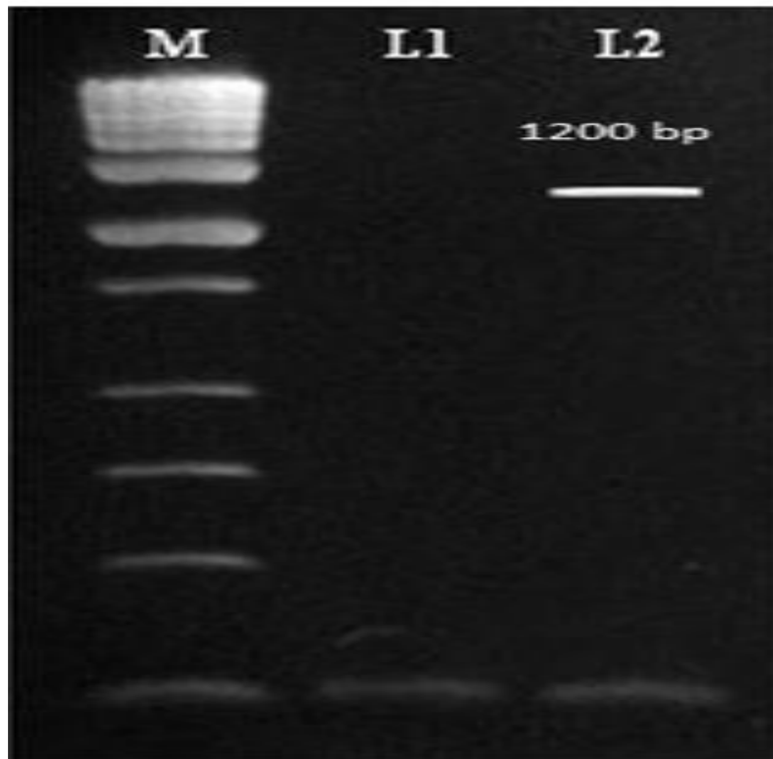

Fig. 4: Nested PCR product obtained with primer pair P1/P7 and nested primer pair R16F2n/R16R2 for phytoplasma detection

3.3: Physiological studies

The physiological responses of plants to phytoplasma infection were estimated as the content of total soluble protein, lipids, carbohydrates, and phenol oxidase in infected and healthy plants. The obtained results in Table (4) indicated that the values of estimated compounds were varied with highly significantly variation among the tested plant parts samples of healthy and infected plants. In general, the total soluble protein was reduced with 90.031 and 59.725% in leaves and buds of infested sesame plants in comparison with healthy plants, respectively. In the same trend, the phytoplasma infection reduced total soluble protein by 45.273 % on infected sesame plants than the healthy plants. In regard to the effect of infection on total lipids, the level was increased in infected leaves with 8.575 %, while it decreased with 87.514 in infected buds than that in healthy ones. In infected sesame plants the total lipids level was reduced by 32.465 % than the healthy ones. In case of the effect of infection on total carbohydrate, the level was increased in infected leaves with 2.780 and 14.622 % in leaves and buds of infected sesame plants than healthy ones. Also, the Phenol oxidase content in infected sesame plants was reduced in infected sesame leaves and buds with 48.569 and 84.435 %, respectively. Generally, it could be concluded that the phytoplasma infection reduced total soluble protein and Phenol oxidase in each of leaves and buds of infected sesame plants. In the contrast, the total lipids were increased in infected leaves and total carbohydrate increased in infected leaves and buds. These results cleared that the infection affected plant resistance by reducing phenol oxidase compound and guide plants to vegetative growth by increasing the carbohydrates and lipids in sesame plants. The obtained results found in harmony with those of [26] who stated the phytoplasmas disturbing complex plant hormone networks, suppress plant immunity and modify plant structure. Also, the phytoplasma infection stress also causes imbalances in the levels of defense-related antioxidants, glutathione, ascorbic acid, reactive oxygen species (ROS), and-in particular-hydrogen peroxide [27]. In the same trend, the reduction in some specific components like flavonoids and phenolic compounds had been also reported [28,29,30]. The results found disagree with those of [31] who reported that soluble protein and phenolics were increased while soluble sugar, hydrogen peroxide and malondialdehyde were decreased in phytoplasma-infected plants.

3.4: Biotechnological studies: Detection of phytoplasmas DNA, RNA in infected and healthy sesame plants

Samples source: The infected and healthy sesame plants samples were collected from Abo Hammad district, Sharkia Governorate, Egypt. All samples were symptomatic as sesame phyllody, except the healthy plant as shown in Fig. (4). Symptoms were recorded and all sampled applied directly for DNA extraction and molecular detection. Molecular Detection: Ethidium bromide-stained gel of PCR amplification products obtained by using universal primer pair P1/P7 in PCR amplification for phytoplasma detection. Also, Nested primer R16F2n/R16R2 was used and confirm the infection with a product size range about 1200 bp for infected sample, while no results were shown in healthy plants (Fig. 4). Ethidium bromide-stained gel of PCR amplification products obtained by using universal primer pair P1/P7 was used for phytoplasma detection. DNA was extracted from sesame plant samples showed symptoms phytoplasma like phyllody. M=1kb DNA marker, L1: Healthy Plant, L2: Infected sesame plant (with phytoplasma like symptoms). The Agarose gel electrophoresis of nested

PCR products from the 16SrRNA gene using primers R16F2n/R16R2 was cleared in Fig. (4). It was observed that some symptoms in sesame are like to phytoplasma symptoms such as, grouping of branches of developing tissues, virescence which is pigmentation of non-green flower parts to green, phyllody, weakness of plants, reddening of leaves and stems generalized yellowing, and phloem necrosis. These symptoms are also observed by [1] who noticed the same symptoms in sesame plants. The observation of phytoplasma was confirmed by using PCR primers P1/P7 and the result was 1.8 kb bands in gel electrophoresis [32]. The PCR method easily distinguishes plant and phytoplasma so that a screening of varieties for the presence of phytoplasma could take place [19,20]. The confirmation step is then done by using nested PCR with specific primers R16R2/ R16F2n, this assay has been widely used for the detection of phytoplasma and is probably the most thoroughly investigated. It detects all strains of phytoplasma whereas healthy plants do not react [33]. Nested-PCR with a combination of different universal primers can improve the diagnosis of unknown phytoplasma present with low titter in the symptomatic host [34]. PCR has been widely used in the detection of many organisms in-clouding viruses and virus like.

4. Conclusions

Finally, polymerase chain reaction (PCR) with degenerate primer P1 / P7 followed by nested primer R16R2/ R16F2n able to identify phytoplasma of infected sesame plants to overcome problems concerned to sensitivity of phytoplasma detection.

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