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EFFECT OF PLANT EXTRACTS AND ESSENTIAL OILS ON *PESTALOTIOPSIS MANGIFERA*; CAUSING BLACK SPOT DISEASE ON MANGO

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ABSTRACT: *Pestalotiopsis mangiferae* the causal organism of Mango fruits black spot, was highly isolated from three governorates in Egypt. Plant extracts of Clove, Camphor, Oleander, Gall, and Garlic, also some essential oils such as clove, mint, garlic, and camphor were tested on the inhibition, growth, and sporulation of *Pestalotiopsis mangifera* both under laboratory and field conditions. Under laboratory condition, clove plant extracts suppressed the growth of *Pestalotiopsis mangiferae* at all tested concentration (10, 15 and 20%). The extracts of garlic and galls had the second and third ranks, in this respect. All tested plant oils reduced the growth of *Pestalotiopsis mangiferae* significantly in comparison with control. Mint oil followed by camphor on had the best efficiency in reducing the fungal growth. Under field condition application of either galls and/or clove oil and clove extracts (5%) gave the best result. Moreover, the average number of healthy fruits per tree recorded 35 and 34, respectively, in response to the application of galls and clove plant extracts. Also, the Shelf-life period of mango fruit; both artificially inoculated with *Pestalotiopsis mangiferae* or not; was increased in response to the application of either plant extracts and/or plant oil; significantly.

Key words: *Pestalotiopsis mangiferae*, Mango fruits black spot, plant extract and essential oils.

INTRODUCTION

Mangoes (*Mangifera indica* L.) are popular, nutritional tropical fruit, belonging to the family of *Anarcadiaceae* which are now one of the most important fruit crops in tropical and subtropical areas of the world. Global exports of mangoes, guavas and mangosteens rose to approximately 2.2 million tons in 2020, an increase of 2.9 percent, or some 60 000 tons, from 2019 (Food and Agriculture Organization of the United Nations 2021). Quality of fresh produce is one of the key factors having significant relationship with the consumer acceptability and marketability; and has always been a major concern of stakeholders from production level to marketing (Shewfelt, 1999).

Several fungal diseases attack mango trees during different growth stages causing considerable losses in mango fruit yield. Microorganism associated with post-harvest spoilage of fruits have engaged the attention of many mycologists for years (Okigbo, 2001).

Postharvest Infection often increases in the store because of the contamination from infected fruits accompanied by wounds resulting from poor harvest, handling or poor storage. this disease development is economically very important and responsible of losses due to diseases infection either under field or store condition thereby limiting its domestic and export marketing (Bally *et al*., 2009) as well as resulting in heavy economic losses (Barkai-Golan, 2001; Narayanasam, 2006).

Mango fruit has also been found prone to postharvest fruit decay due to rapid disease development during storage and ripening (Prusky et al., 2009). Anthracnose (caused by *Colletotrichum gloeosporioides*) is regarded one of the major postharvest diseases of mango (Bally et al., 2009). Stem end rot and black spots (*Alternaria alternata*, *Pestalotiopsis mangifera*, *Botryodiplodia Theobroma* and *Colletotrichum gloeosporiodes*) and soft rot of fruits, caused by *Asperigillus niger* and *Rhizopus stolonifera*. which also been reported to cause significant postharvest decay in mango (Prusky et al., 2009)

Antimicrobial chemicals such as fungicides are often used in control of plant diseases, thus,

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The concerns about chemical residue in the environment and the development of resistance by the pathogen (Spotts and Cervantes 1986; Osuinde *et al*. 2001). Under this concept, all possible modes of plant pests and disease control methods should be integrated to minimize the excessive use of synthetic pesticides.

This concerns leads the scientists to search about the nonchemical approaches to postharvest disease control (Wilson *et al*., 1987). There are many records that some plant species indicated pharmacological and biological activity such as antimicrobial activity and fungicidal properties depended on various plant products including oils, alkaloids, resin, saponin, organic acids and gums (Cowan, 1999). The importance of essential oils in crop protection is being increasingly recognize under the concept of Integrated Pest and Disease Management (IPDM).

In this study we tested many alternative methods such as biological control agents against, plant extracts and essential oils on the most frequent post-harvest pathogens associated with mango fruits *in vitro* and *in vivo*, to avoid the hazards of using the fungicides to control these pathogens which have been isolated from the infected fruit of mango and recorded in many areas around the world. However, the survey of the common and frequent air borne pathogens attacking mango fruit seems to be very important to be carried out in the Egyptian mango farms. Consequently, samples of infected fruits should be collected from different mango cultivated areas in Egypt. The associated fungi should be isolated, purified, and identified.

MATERIALS AND METHODS

1. Survey of the diseases in different governorates of Egypt:

A survey was carried out during (2018 and 2019) seasons to determine the occurrence of infection with different diseases of mango from flowering to harvest stage and storage in different governorates in Egypt i.e, Aswan, Sharqia, Behira and Menoufia.

The samples were collected during harvest season of mango. Disease incidence (DI) was recorded according to the presence or absent of symptoms of the fruit parts of mango tree. The percentage of disease incidence (PDI) was calculated using the following formula:

$$\text{PDI} = \frac{\text{Number of diseased fruits}}{\text{Total number of sampled fruits}} \times 100$$

2. Isolation, Purification and Identification of the causal organisms:

A. Isolation of the causal organisms:

Postharvest diseases of mango fruits showing clear symptoms (especially, brown – black spots) and degradation symptoms on fruit because of fungus activities were collected from different governorates of Egypt. The samples were packed and stored at 10°C and humidity 90% to 95% for two weeks. The infected fruit showed symptoms were washed by running tap water to remove dust adhesive particles. The samples were surface sterilized by 70% ethanol, rinsed serval times with sterilized distilled water, dried between sterilized filter papers, cut into small pieces from spots with apart of the healthy tissue and then planted on PDA medium amended with antibacterial antibiotic (20 ppm streptomycin sulphate).

Typical formula per liter:

Potatoes	200 g	Agar	15 g
Dextrose	20 g	pH was adjusted at 5-6	

Petri dishes were incubated at 25°C and examined daily for the fungal growth.

B. Purification of the isolated microorganism:

Purification of the isolated fungi was carried out using hyphal tips and/or single spore culture techniques according to Dhingra and Sinclair (1977).

C. Identification of isolated microorganisms:

According to the morphological and physiological aspects of the obtained isolates; they were primarily identified at Botany Department, Faculty of Agriculture, Menoufia University. Verification of identification was carried out at the Department of Mycological

Researches, Plant Pathology Institute (ARC), Giza, Egypt.

3. Pathogenicity test experiments:

Pathogenicity test experiments were conducted on mango cultivars Fajr Kelan, Keitt, Kent and Naomy using the obtained isolates individually. Mango fruits were washed by tap water, surface sterilized by 70% ethanol, gently wounded using fungal needle and sprayed with each isolate and stored at room temperature (15 – 20°C). The average diameter of the abundant spots were calculated after 3, 6 and 9 days of inoculation. Three fruits were used as replicates for each treatment.

4. Laboratory experiment:

A complete randomized design with three replicates was following in this experiment.

IV.1. Effect of plant extracts on linear fungal linear growth:

IV.1.1. Preparation of plant extracts:

Two hundred grams of each tested plant (Table 1) were soaked in 1000ml sterilized distilled water for 24 hours. The obtained extracts were separately heated at 90°C for 30 minutes, then filtered through filter paper, completed to 1 liter and autoclaved at 90°C for 60 minutes. The concentration of 10, 15 and 20% were obtained into PDA medium according to the formula.

$C1 \times V1 = C2 \times V2$

C1 ⟶ more concentration solution.

V1 ⟶ volume needed for a more concentration solution.

C2 ⟶ final concentrated solution.

V2 ⟶ desired volume for the final solution however, control treatment had PDA medium only.

IV.2. Effect of some essential oils on the fungal linear growth:

Crude oils of clove, mint, garlic and camphor were obtained from El-Gomhoria Company, Egypt. The oils were emulsified with 3% (v: v) tween 20. The emulsified oils were separately mixed with PDA medium to obtain the concentrations of 5, 10 and 15%. Control treatment received tween 20 mm at the used concentration. Different volumes of either essential oil or plant extract were mixed with the sterile PDA to obtain various concentration. The supplemented PDA were individually inoculated with agar disc (5 mm in diameter) of *Pestalotiopsis mangifera*, *Fusarium sp*. and *Alternaria altrernata* isolated pathogens (from 7 days-old PDA cultures) and incubated at 25 + 2°C for 9 days then the blocking fungal development was calculated.

1. Field experiment:

These experiments were carried out for successive seasons at Al-Sheble Farm, Badr district, Behira governorate using mango cultivar keitt. Mango trees were sprayed by different plant extracts (Table 1) at the concentration of 10%. However, control treatment didn't spray at all. The same methods were followed for plant oils at the concentration of 5%. Three replicates were used for each treatment and the applications were repeated every week during season the treatment. During flowering stage disease incidence was recorded as number of final healthy fruit obtained after June drop and before harvest in relation to the total number of flowers.

$$\text{Disease index (DI)} = \frac{\text{number of final healthy fruit} - \text{total number of flowers}}{\text{total number of flowers}} \%$$

Table (1). Medicinal and ornamental plants used for extraction.

English name	Scientific name	Used part
Clove	*Syzygium aromaticum*	Fruits
Camphor	*Eucalyptus rostrate*	Leaves
Oleander	*Nerium oleander*	Leaves
Gall	*Quercus infectoria*	Fruits
Garlic	*Allium sativum*	Cloves
Mint	*Mentha arvensis*	Leaves

2. Store experiment:

Healthy mango fruits (400 – 500 g/each) keitt cultivar were collected, washed by tap water, left for water evaporation and surface sterilized using 70% ethanol. Under sterilized condition a circles of 4 cm diameter on the fruit were gently wound using fungal needle and achieved artificial infection by applying a 4 mm diameter disc of *Pestalotiopsis mangifera* mycelium. The fruits were sprayed by different plant extracts like clove, galls, garlic, mix of them, garlic oil and clove oil at the concentration 10%. The diameter of upended were estimated after 4, 8 and 12 days of inoculation.

On the other hand, mango fruit (the same quality) were washed and sterilized as mentioned before and treated with the same treatments (without inoculation) for estimating shelf life of the fruit. The fruits were stored under room temperature during winter season (15 – 25°C).

3. Shelf-life experiment:

Healthy mango fruits (400 – 500 g/each) keitt cultivar were collected at the same maturity (stage 2), washed by tap water, left for water evaporation and surface sterilized using 70% ethanol. Under sterilized condition a circles of 4 cm diameter on the fruit were gently wound using fungal needle and achieved artificial infection by applying a 4 mm diameter disc of *Pestalotiopsis mangifera* mycelium. The fruits were sprayed by different plant extracts like clove, galls, garlic, and mix of them, garlic oil and clove oil at the concentration 10%. The diameter of upended were estimated after 4, 8 and 12 days of inoculation. Brix (sugar content) in the fruits were estimated after 4, 8 and 12 days of inoculation by REFRACTOMETER. Mango fruits of four ripening stages (RS) were determined by pulp color. Stage 1, representing mango with yellow pulp color area of 0 – 10%; Stage 2, 11 – 40%; Stage 3, 41 – 70% and Stage 4, 71 – 100% yellow color. On the other hand, mango fruit (the same quality) were washed and sterilized as mentioned before and treated with the same treatments (without inoculation) for estimating shelf life of the fruit. The fruits were stored under room temperature during winter season (15 – 25°C).

RESULTS

I. Isolation of causal organism:

Samples of diseased mango fruit were collected from six district belong to four Egyptian governorates i.e., Aswan, Behera, Ismailia and Menoufia Table (2). The obtained results clear that *Pestalotiopsis mangiferae* didn't recorded in Aswan fruits and was highly obtained from Behera and Menoufia samples. Such fungus recorded 25, 35.2 and 36%, respectively at Wadi El-Natron, Nubaria and Badr restricts (Behera governorate). However, at Wadi El-Molak; Ismailia governorate *Pestalotiopsis mangiferae* recorded 25% of the diseased samples and 23.3% of those obtained from Sadat city; Menoufia governorate.

Table (2). Survey of the isolation fungi from mango fruits obtained from different Egyptian governorates.

Governorate	District	No. of samples	*Pestalotiopsis mangifera*		*Alternaria alternata*		*Fusarium sacchari*		*Lasiodiplodia theobromae*	
			No. of fruit	DI%	No. of fruit	DI%	No. of fruit	DI%	No. of fruit	DI%
Aswan	Garf hussin	10	0	0	0	0	0	0	2	20
Behera	Wadi Elnatron	20	5	25	3	15	2	10	1	5
	Nubaria	17	6	35.2	5	29.44	3	17.64	2	11.76
	Badr	25	9	36	7	28	4	16	1	4
Ismailia	Wadi Elmolak	12	3	25	4	33.33	2	16.66	0	0
Menoufia	Sadat City	30	7	23.3	10	33.33	6	20	2	6.66

II. Cultivar susceptibility to the different diseases:

Results present in Table (3) clear that all tested mango cultivar fruits artificially inoculated with each pathogen are susceptible to the infection with the three diseases. No significant differences were noticed between the cultivars toward *Fusarium sacchari* and *Alternaria alternata* isolates, However, Kent cultivar showed less significant susceptibility to *Pestalotiopsis mangiferae* isolate compared to Fajrkelan, Keitt and Naomy mango cultivars the average diameter of the appeared spot-on Kent fruit was 2.3 cm after 12 days of incubation with *P. mangiferae* while these were 3.2, 3.2 and 2.5 on Fajrkelan, Keitt and Naomy fruits, respectively.

III. Laboratory experiment:

III.A.1. Effect of different plant extracts on the linear growth of *Pestalotiopsis mangiferae*:

Results present in Table (4) indicate that after three days, clove plant extract was the best one for reducing the growth of *P. mangiferae* fungus. It suppressed the fungal growth completely even at the minimum used concentration (10%). Garlic plant extract came in the second rank where it reduced the fungal growth significantly at 10% concentration and suppressed the growth completely at 15 and 20% concentration. Galls had the third rank where growth suppression was only obtained at 20% concentration. Those three extracts showed significant differences in reducing the fungal growth while the other tested plant extracts (Camphor, Mint and Oleander) reduced the fungal growth, but insignificantly. However, after six days of inoculation; clove and garlic extract also showed growth suppression of *P. mangiferae* at all used concentrations at 20% only. The same results were obtained after 9 days of incubation. It was noticed that camphor (10%), garlic (10%), oleander (10%) and mint (10, 15 and 20%) extracts gave the same results of control treatment and didn't affect the fungal growth.

III.A.2. Effect of some plant oils on the linear growth of *P. mangiferae*:

Results shown in Table (5) clear that all tested plant oils reduced the growth of *P. mangiferae* significantly in comparison with control. Mint oil, at all concentrations gave the best results at all time of investigation. It completely suppressed the fungal growth at 10 and 15% concentrations after 3.6 and 9 days of inoculation. Camphor plant oil had the second rank of efficiency on reducing the fungal growth. However, garlic oil was the least effective one in reducing *P. mangiferae* fungal growth. Increasing the concentration of any tested oil was more effective in reducing the growth and vice versa.

IV. Field experiments:

Results present in Tables (6 and 7) indicate that galls and clove extracts (10%) showed the best results of number of inflorescences, fruit setting and number of fruits remained after June drop. The average number of fruits per tree were 35 and 34 respectively for the application of galls and clove extracts. However, plant oils showed the least efficiency where garlic oil and clove oil (5%) resulted 1.3 and 1.7 fruits/tree which were the least obtained results at the second season.

Table (3). Cultivar susceptibility to the different diseases.

Fungi / Cultivar	*Fusarium sacchari*	*Alternaria alternata*	*Pestalotiopsis mangiferae*
Fajrkelan	2.4[a]	2.3[a]	3.2[a]
Keitt	2.2[a]	2.3[a]	3.2[a]
Kent	2.1[a]	2.1[a]	2.3[b]
Naomy	2.4[a]	2.6[a]	2.5[ab]
L.S.D 0.05	0.98	1.50	0.74

Table (4). Effect of plant extracts on the linear growth of *P. mangiferae*.

Plant extract	Conc. (%)	Linear growth (cm)		
		Day (3)	Day (6)	Day (9)
Camphor	10	3.42^{abc}	7.67^{abc}	9.00^{a}
	15	3.42^{abc}	7.17^{abc}	9.00^{a}
	20	2.92^{c}	6.83^{de}	8.83^{a}
Clove	10	0.00^{e}	0.00^{i}	0.00^{g}
	15	0.00^{e}	0.00^{i}	0.00^{g}
	20	0.00^{e}	0.00^{i}	0.00^{g}
Galls	10	1.73^{d}	3.13^{f}	5.27^{d}
	15	1.13^{d}	2.33^{g}	3.50^{e}
	20	0.00^{e}	0.83^{h}	1.87^{f}
Garlic	10	1.92^{d}	6.16^{e}	9.00^{a}
	15	0.00^{e}	2.83^{fg}	6.50^{c}
	20	0.00^{e}	0.00^{i}	0.00^{g}
Mint	10	3.92^{ab}	8.08^{a}	9.00^{a}
	15	3.92^{ab}	7.92^{ab}	9.00^{a}
	20	3.25^{bc}	7.17^{bcd}	9.00^{a}
Oleander	10	4.25^{a}	7.83^{ab}	9.00^{a}
	15	3.83^{ab}	7.17^{bcd}	8.83^{a}
	20	3.33^{bc}	6.92^{cd}	8.33^{b}
Control		3.50^{abc}	6.93^{cd}	9.00^{a}
L.S.D 0.05		0.78	0.68	0.44

Table (5). Effect of some plant oils on the linear growth of *P. mangiferae*.

Plant oil	Conc. (%)	Linear growth (cm)		
		Day (3)	Day (6)	Day (9)
Camphor	5	0.83^{c}	2.33^{d}	3.90^{d}
	10	0.00^{d}	0.67^{e}	2.23^{e}
	15	0.00^{d}	0.00^{e}	0.00^{f}
Clove	5	0.80^{c}	2.60^{cd}	3.67^{d}
	10	0.73^{c}	2.23^{d}	3.33^{d}
	15	0.00^{d}	0.00^{e}	0.00^{f}
Garlic	5	2.20^{b}	4.00^{b}	6.00^{b}
	10	2.03^{b}	4.00^{b}	5.17^{bc}
	15	1.80^{b}	3.40^{bc}	4.33^{cd}
Mint	5	0.47^{cd}	1.00^{e}	1.43^{e}
	10	0.00^{d}	0.00^{e}	0.00^{f}
	15	0.00^{d}	0.00^{e}	0.00^{f}
Control		3.50^{a}	6.93^{a}	9.00^{a}
L.S.D 0.05		0.47	0.90	1.00

Table (6). Effect of plant extracts, plant oils on mango flowering and fruit setting at first season.

Foliar spray				
Treatment	**Conc.%**	**No.of Inflorescence**	**Fruit setting**	**Average no. of fruits/tree**
Clove extract	10	71.7[a]	46.0[a]	34.0[a]
Galls extract	10	72.3[a]	45.0[a]	35.0[a]
Garlic extract	10	45.3[b]	33.7[ab]	21.7[bc]
Garlic oil	5	24.0[c]	7.0[c]	1.3[d]
Clove oil	5	13.0[c]	7.3[c]	1.7[d]
Control (–)		83.7[a]	30.7[b]	16.7[c]
L.S.D 0.05		15.7	13.0	9.5

Table (7). Effect of plant extracts, plant oils on mango flowering and fruit setting at second season.

Foliar spray				
Treatment	**Conc.%**	**No.of inflorescence**	**Fruit setting**	**Average no. of fruits/tree**
Clove extract	10	116.3[a]	69.3[a]	6.7[abc]
Galls extract	10	124.3[a]	67.3[a]	13.0[ab]
Garlic extract	10	51.3[bc]	18.3[ab]	15.3[a]
Garlic oil	5	35.3[c]	9.3[b]	2.0[bc]
Clove oil	5	96.0[ab]	61.0[ab]	0.67[bc]
Control (-)		36.0[c]	21.7[ab]	0.0[c]
L.S.D 0.05		51.9	49.9	11.7

At first growth season, garlic plant extracts (5%) gave the best efficiency of the average number of healthy fruits after June drop (15.3/tree). This was followed by galls and clove extracts (5%) which resulted 13 and 6.7 fruits/tree, respectively.

Results of both seasons indicate that the application of plant extracts gave the best results of fruit setting and healthy fruits per tree. However, plant oils gave the least efficiency of flowering, fruit setting and healthy fruit/tree.

V. Store experiment:

V.1. Effect of some plant extracts and oils on the severity of infection with *Pestalotiopsis mangiferae:*

Nearly equal size of mango fruits (keitt cultivar) in were artificially inoculated with *Pestaiotiopsis mangiferae* fungus. Such fruits were separately treated with plant extracts i.e., clove, galls, garlic and mixture of them at the concentration of (10%) and garlic oil & clove oil (10%).

Disease severity was estimated after 4, 8 and 12 days of inoculation. Results shown in Table (8) and clear that all treatments with the plant extracts and oils significantly decreased the severity of infection with disease compared to control, after 4 days. However, four days later, plant extracts showed significant reduction in disease incidence compared to control, but plant oil didn't. After twelve days of inoculation, all the treated fruits with either plant extracts or oil showed significant disease reduction in comparison with control ones.

V.2. Effect of some plant extracts and oils on the severity of non-infection fruits:

Healthy, equal size and the same ripeness stage mango fruit (Keitt cultivar) were individually treated with either plant extracts or plant oil. Such fruits were left at room temperature (20 – 22°C) and examined every four days to estimate post-harvest incidence. Control fruits were treated with sterilized stilled water. The obtained results clear that all tested treatments significantly reduced the incidence of mango fruit disease up to 8 days in comparison with control Table (9). The best result was obtained when the extracts of clove, galls and their mixture garlic one was applied, separately. The same result was obtained when clove oil was applied. In the abovementioned treatments mango fruits still free of any infection up to 20 days. Application of garlic plant extract alone showed the least efficiency of mango shelf life.

Table (8). Effect of some plant extracts and oils on the severity of infection with *Pestalotiopsis mangiferae*.

Infected fruits				
Treatment	**Conc. %**	**Day 4**	**Day 8**	**Day 12**
		Mean	**Mean**	**Mean**
Clove extract	10	1.3^{b}	1.7^{b}	2.0^{b}
Galls extract	10	1.5^{b}	1.7^{b}	1.8^{b}
Garlic extract	10	1.4^{b}	1.6^{b}	2.0^{b}
Mix	10	1.5^{b}	1.6^{b}	1.9^{b}
Garlic oil	10	1.6^{b}	1.8^{ab}	1.8^{b}
Clove oil	10	1.6^{b}	2.1^{ab}	2.1^{b}
Control		2.2^{a}	2.3^{a}	3.2^{a}
L.S.D 0.05		0.54	0.56	0.95

Table (9). Effect of some plant extracts and oils on the severity of non-infection fruits.

Non-Infected fruits				
Treatment	Conc.%	Day 4	Day 8	Day 12
		Mean (cm)	Mean (cm)	Mean (cm)
Clove extract	10	0.00^{b}	0.00^{c}	0.00^{c}
Galls extract	10	0.00^{b}	0.00^{c}	0.00^{c}
Garlic extract	10	0.00^{b}	0.15^{b}	0.23^{b}
Mix	10	0.00^{b}	0.00^{c}	0.00^{c}
Garlic oil	10	0.00^{b}	0.17^{b}	0.18^{b}
Clove oil	10	0.00^{b}	0.00^{c}	0.00^{c}
Control		0.20^{a}	0.35^{a}	0.57^{a}
L.S.D 0.05		0.03	0.05	0.07

Table (9). Effect of plant extracts and plant oils on the severity of infection with *Pestalotiopsis mangiferae* (mm^2) in relation to sugar content (Brix), stage of ripeness and shelf life.

Infected fruit				
Treatment	**Zone area (mm^2)**	**Brix %**	**Shelf life (days)**	**Stage of ripeness**
Clove extract	25.3^{ab}	19.1^{a}	4.3^{abc}	3.3^{ab}
Galls extract	27.3^{ab}	18.9^{a}	5.0^{ab}	3.0^{b}
Garlic extract	30.3^{ab}	20.8^{a}	2.7^{bc}	4.3^{a}
Mix	21.0^{ab}	17.6^{a}	6.3^{a}	3.0^{b}
Garlic oil	18.3^{b}	19.7^{a}	6.3^{a}	3.0^{b}
Clove oil	20.7^{ab}	17.7^{a}	5.3^{ab}	3.0^{b}
Control	32.0^{a}	19.9^{a}	1.3^{c}	4.0^{ab}
L.S.D 0.05	13.5	3.5	3.6	1.1

V.3. Shelf life of Keitt mango cultivar as affected by application of some plant extracts and oils:

Results shown in Table (9) clear that increasing sugar content in the fruit led to the increment of severity of infection with *Pestalotiopsis mangiferae* both artificially inoculated fruits or non-inoculated .application of plant oils increased the time of shelf life, and the best action was obtained when garlic oil was applied. mixture of plant extracts gave the same result (6.3 days) + 12 days before estimation). shelf life of the non-infected fruits was much higher than those of in artificially inoculated ones. Application of any tested plant extracts and/or plant oils increased shelf life of mango fruits significantly; except that of garlic extract (5%) the best action was achieved when galls plant extract was applied followed by clove oil and extract (Table 10).

V.4. Effect of plant extracts and/or plant oil on the loss of mango fruit weight (Keiit cultivar):

Mango fruits (Keitt cultivar) in were artificially inoculated with *Pestalotiopsis mangiferae* fungus. Such fruits were separately treated with plant extracts i.e., clove, galls, garlic and mixture of them at the concentration of (10%), garlic oil and clove oil (10%). Result present in Table (11) indicate that application of garlic oil and/or garlic plant extracts (10% per each) gave the best result of weight loss of mango fruit up to 12days of estimation. Clove oil and clove extracts came in the second rank of efficiency. On the other hand, the most lose weight were observed on the non-treated fruits and those treated with the mixture of plant extracts. Lose weight of the non-infected fruits was less than those of in artificially inoculated ones. Garlic plant extracts and/or garlic oil (10% per each) gave the best result of weight loss of mango fruit up to 12 days of estimation. Clove extracts and clove oil came in the second rank of efficiency. On the other hand, the most lose weight were observed on the non-treated fruits and those treated with the mixture of plant extracts in Table (12).

Discussion

Pestalotiopsis mangiferae was highly isolated from mango fruits obtained from Behera and Menoufia Governorates but didn't isolated from Aswan samples. These result could be due to the environmental conditions especially temperature and RH as mentioned by Verma and Tirath Singh (1996), who studied the mode of survival of this fungus. *Pestalotiopsis mangiferae* is the causal agent of grey mould disease and it over summered in acervuli and necrotic spot as dormant mycelium. Such results were reported by Mordue (1980), Verma *et al*. (1991), Johnson *et al*. (1992),

Verma and Tirath Singh (1996), Ploetz *et al*. (1996), Hu Meijiao *et al*. (2012), Humaira Rizwana *et al*. (2012) and Asma Rashid *et al*. (2013). However, Ko *et al*. (2007) conducted pathogenicity test of this fungus and observed the symptoms on all inoculated leaves and reisolated the fungus from them. They also mentioned that the un inoculated leaves remained completely free from symptoms.

Under laboratory conditions, clove plant extracts suppressed the growth of *Pestalotiopsis mangiferae* at all tested concentrations (10, 15 and 20%). In this respect, the extracts of garlic and galls had the second and third ranks. However, plant extracts were efficiently used to control the growth of many pathogenic fungi by Kumar *et al*. (2006), Chavada and Chaudhari (2015), Beena *et al.* (2017), Devi and Devi (2017), Miya and Shamim (2017), Sarkhosh *et al.* (2018) and Shiriki *et al*. (2019). Camila *et al*, 2018 tested a different aqueous plant extracts such as the clove extract, they found clove extract effects on the germination and initial development of lettuce seeds were observed, also the effect of clove extract affected on the mycelial growth of the fungus *Cercospora longissima* (isolated from lettuce plants) were evaluated.

Table (10). Effect of plant extracts and plant oils on the severity of non-infection fruits in relation to sugar content (Brix), stage of ripeness and shelf life.

Non-Infected fruit				
Treatments	**Total spot area (mm^2)**	**Brix %**	**Shelf life (days)**	**Stage of ripeness**
Clove extract	00.0^{c}	16.2^{d}	10.0^{a}	3.0^{b}
Galls extract	00.0^{c}	16.5^{d}	10.7^{a}	3.0^{b}
Garlic extract	23.3^{b}	20.0^{ab}	4.7^{b}	4.3^{a}
Mix	00.0^{c}	16.4^{d}	12.7^{a}	3.0^{b}
Garlic oil	18.3^{b}	18.8^{bc}	6.0^{b}	4.0^{a}
Clove oil	00.0^{c}	17.8^{c}	10.3^{a}	3.0^{b}
Control	46.7^{a}	20.7^{a}	5.3^{b}	4.3^{a}
L.S.D 0.05	10.9	1.2	3.7	0.6

Table (11). Effect of different plant extracts and plant oils on loss of mango fruits weight (infected with *Pestalotiopsis mangiferae*).

Infected fruits							
Treatment	**Total weight of mango fruit (Gram)**	**PLW* % -days after harvest**					
		Day 4		**Day 8**		**Day 12**	
		Weight (gram)	**PLW%**	**Weight (gram)**	**PLW%**	**Weight (gram)**	**PLW%**
Clove	4910	4672	(4.84)	4558	(7.16)	4420	(9.97)
Clove oil	3998	3811	(4.67)	3721	(6.92)	3605	(9.82)
Galls	4830	4607	(4.61)	4458	(7.70)	4317	(10.62)
Garlic	4350	4183	(3.83)	4129	(5.08)	3985	(8.39)
Garlic oil	5310	5097	(4.01)	4968	(6.44)	4873	(8.22)
Mix	5113	4865	(4.85)	4744	(7.21)	4520	(11.59)
Control	4890	4620	(5.52)	4425	(9.50)	4304	(11.98)

* PLW% post-harvest lost weight %.

Table (12). Effect of different plant extracts and plant oils on loss of mango fruits weight (non-infected fruit).

Non infected fruits							
Treatment	**Total weight of mango fruit (gram)**	**PLW* % -days after harvest**					
		Day 4		**Day 8**		**Day 12**	
		Weight (gram)	**PLW%**	**Weight (gram)**	**PLW%**	**Weight (gram)**	**PLW%**
Mix	4401	4278	(2.79)	4184	(4.93)	4063	(7.68)
Galls	4392	4258	(3.05)	4164	(5.19)	4076	(7.19)
Clove	3615	3517	(2.71)	3452	(4.50)	3380	(6.50)
Clove oil	4209	4108	(2.39)	3996	(5.06)	3907	(7.17)
Garlic	3033	2980	(1.74)	2937	(3.16)	2897	(4.48)
Garlic oil	2440	2393	(1.92)	2351	(3.64)	2312	(5.24)
Control	4332	4187	(3.34)	4046	(6.60)	3922	(9.46)

* PLW% ⟹ post-harvest lost weight %.

All tested plant oil reduced the growth *Pestalotiopsis mangiferae* significantly in comparison with control. Mint oil followed by camphor one had the best efficiency in reducing the fungal growth. Arina and Ahmad 2002, found that the toxicity of clove oil effective on the germination and growth lysis of conidia and inhibition of mycelial growth were detected. Also (Abhishek Sharma *et al.* 2017) found that most active being the clove oil, exhibiting complete inhibition of mycelial growth and spore germination at 125 ppm. Also, they were identified by gas chromatography–mass spectroscopy analysis. The major components were eugenol (75.41%), E-caryophyllene (15.11%), α-humulene (3.78%) and caryophyllene oxide (1.13%). Effect of clove oil on surface morphology of *F. oxysporum* f. sp. *Lycopersici*.

Application of such botanical extracts and/or oils to control mango post-harvest diseases are very important to avoid the use of chemical fungicides both for the local consumption and exportation. Fruit of the treated plants with either plant extracts or oils were exported to different companies of Netherland and Lebanon as organic production after their examination, in addition to the positive obtained results of disease management, shelf life of mango fruit was significantly extended in response to the application of either plant extracts and/or oils. This finding is very important for the local marketing and exportation. Application of garlic oil and/or garlic plant extracts to mango fruit (Keitt cultivars) led to decrease of the loss weight of fruit up to 12 days of storage, both inoculated with *Pestalotiopsis mangiferae* or not. It was noticed that such application (s) led to fruit ripeness.

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دراسة تأثير بعض المستخلصات النباتية والزيوت العطرية على فطر *Pestalotiopsis mangifera* المسبب لمرض البقعة السوداء في المانجو

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الملخص العربى

تتعرض ثمار المانجو للإصابة بالعديد من الأمراض الفطرية بعد الحصاد، وفي هذه الدراسه تم عزل فطر Pestalotiopsis managiferae المسبب لمرض Black spot من عينات ثمار المانجو المتحصل عليها من محافظات البحيرة والشرقية والمنوفية في حين لم توجد مثل هذه المسببات في عينات محافظة اسوان. وتحت ظروف المعمل أدت المستخلصات النباتيه والزيوت النباتيه في نقص معنوي في الفطريات المعزوله. وكان زيت النعناع متبوعا بزيت الكافورهما الأفضل تأثيرا علي نموالفطرPestalotiopsis managiferae في. وأظهرت الدراسة أن جميع أصناف المانجو المختبرة كانت قابلة للإصابة بالفطر محل الدراسة. هذه الدراسه تمت تحت ظروف الحقل أدت المعاملة بمستخلص العفص وكذلك زيت القرنفل أو مستخلصه النباتي إلي نتائج أفضل في ظهور النورات ،وعقد الثمار وعدوي الثمار السليمة المتبقية علي الشجرة بعد تساقط يونيه .وبالعدوي الصناعية بالفطر Pestalotiopsis managiferae تم المعامله بتركيز ١٠٪بالمستخلصات النباتيه والزيوت (٥٪) وأظهرت النتائج نقص معنوي في حدوث الإصابة في الفطر .وأظهرت الدراسه زيادة مده تخزين ثمار المانجو كإستجابه للمعامله بالمستخلصات النباتيه وكذلك الزيوت المختبرة كما أن مستخلص نبات الثوم وكذلك زيته أظهر تأثيرا إيجابيا في تقليل الفاقد من وزن الثمار المخزنة.