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Thermotherapy of Sunflower Seeds Controlling Charcoal-Rot Caused by *Macrophomina phaseolina*

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Various fungi were isolated from sunflower seed samples collected from two fields in Ismailia and Beni-Suef governorates. Fifteen fungal species belonging to 10 genera were isolated from Beni-Suef fields and thirteen fungal species belonging to 8 genera were isolated from Ismailia field. *Alternaria alternata* recorded the highest frequency from sunflower seed samples collected from the two locations followed by *Aspergillius flavus* and *A. niger*. *Macrophomina phaseolina* was the main seed-borne pathogen isolated from sunflower seeds collected from the two locations followed by *Fusarium oxysporum* and *Fusarium* spp. Applying thermo-treatments through microwave radiation in five exposing periods (15, 30 sec, 1, 2 and 4 min.) and hot water treatment at five soaking periods (30 sec, 1, 2, 4 and 8 min.) gave significant impacts on the occurrence of seed-borne pathogen *M. phaseolina* in sunflower seeds as well as seed germination. Moreover, increasing the exposure time of microwave radiation or soaking in hot water led to enhancing their effects. Soaking in hot water negatively affected the frequency of *M. phaseolina* than microwave radiation treatment. Generally, the obtained results clearly show that microwave radiations and hot water treatments significantly decreased the incidence of pre- and post-emergence damping-off. Moreover, sunflower seeds exposed to either treatment then planted under greenhouse conditions in infested and non-infested soil with *M. phaseolina* or under open field conditions, naturally infested, led to increase of sunflower seed yield. In this respect, the highest impact was obtained when sunflower seeds were soaked in hot water at 2 min followed by soaking in hot water for 1 min and exposure to microwave radiation for 1 min. Data also proved that the efficiency of some thermal treatments was approximately close to efficiency of fungicide in reducing damping-off and charcoal rot diseases during 2017 and 2018 trail seasons, where soaking in hot water for 2 min. was the closest one.

Keywords: Sunflower, charcoal-rot, seed-borne, thermo-therapy, microwave, hot water, fungicides.

Sunflower (*Helianthus annuus* L.) is one of the major oilseed crops grown for edible oil in the world (Weiss, 2000). In Egypt, there are great efforts for increasing the devoted area for its cultivation and production of edible oil. Sunflower is prone to be attacked by a variety of soil- and seed-borne fungal pathogens. Phytopathogenic microorganisms may also affect seeds before or after emergence causing many diseases such as damping off, seedling blight, root rot and stem rot (Mathur and Manandhar, 2003 and Sangawan *et al.,* 2005).

Macrophomina phaseolina Tassi (Goid) was reported as one of the most destructive soil- and seed-borne fungal pathogens affecting sunflower in Egypt and worldwide. It causes charcoal-rot not only on sunflower but also on more than 500 plant species throughout the world and surviving for up to 15 years in the soil as a saprophyte (Purkayastha *et al.*, 2006; Aboshosha *et al.,* 2007 and Ghonim *et al.*, 2014). Charcoal-rot is a serious sunflower destructive disease causing seedling blight, damping-off, root rot and/or basal stem rot (Khan, 2007). In Egypt, *M. phaseolina* has been reported more frequently from sunflower seeds causing charcoal rot (Ibrahim, 2006; Khalil *et al.,* 2014 and Ghonim *et al.,* 2014). The disease decreased sunflower grain yield by 41-79 % (Khan *et al*. 2005) and seed weight by 30-46 % (Gulya, 2002).

Thermotherapy is defined as the application of heat to plant propagation materials or plant parts using specific temperature-time regimes to damage or kill the pathogen without causing significant harm to the host (Allison, 2014 and Araújo *et al.,* 2016). Thermotherapy is also considered as an option for organic growers for seed disinfection and an alternative to the more commonly used chemical controls (Tinivella *et al.,* 2005). The ability to destroy microbes within the seed is something that cannot be achieved with most chemical treatments. The application of heat for seed treatment has been conducted in various forms, including hot water, hot dry air, aerated steam, hot oil soak, microwave radiation and several other derivations of these heat treatments (El-Abbasi, 2004; Forsberg, 2004; Jahn *et al.,* 2006 and Allison, 2014).

Microwave radiation has been utilized for control of seed-borne and seed production (El-Abbasi, 2004; Allison, 2014 and Friesen *et al.,* 2017). Microwave radiation is believed to use heat as the lethal mode of action for controlling pathogens (Reddy *et al.,* 1998; Kraus and Fleisch, 1999). The application of microwave radiation for seed treatment was benefited for controlling rice seed-borne fungi (El-Abbasi, 2004) and dry bean seed to control *Alternaria alternata, Fusarium* spp. and *Penicillium* spp. (Tylkowska *et al.,* 2010).

The present study aimed to determine the frequency and occurrence of *M. phaseolina* from sunflower seed samples in Egypt, and also to throw a beam of light on some advantages of seed thermotherapies as safe alternative comparing to chemical control of charcoal-rot.

Materials and Methods

1- Sample collection:

Twenty seed samples of sunflower were collected from plants growing in two fields located in two different counties of Beni-Suef and Ismailia governorates. Each sample was collected from 20 sunflower plant heads distributed in five stripes and taken while walking in zigzag pattern pathway in the field. Collected heads were air dried, threshed and the seeds were cleaned and then placed in a labeled envelope until testing.

2- Occurrence of sunflower seed-borne fungi:

Detection of seed-borne fungi was done using the standard blotter method according to Anon. (2014). A total number of 400 seeds representing each sample were used. The occurrence percentage of each fungal species was calculated and recorded. Surface-sterilized (immersed into 1% NaOCl for 3 min.) and non–sterilized seeds were plated in 9 cm diam. sterile Petri dishes containing three layers of sterile blotter moistened with sterilized tap water. Ten seeds were placed in each Petri dish and incubated at 26±2^{o}C for 7 days under alternating cycles of 12 h cool white fluorescent light and 12 h darkness. Hyphal tip technique was followed to obtain pure cultures. Pure cultures of all fungi were maintained on potato carrot agar slants for further studies. Fungal identification was carried out depending on their cultural properties, morphological and microscopic characteristics.

3- Preparation of fungal inoculum:

Inoculum of *Macrophomina phaseolina* was prepared using sorghum - coarse sand - water (2:1:2 v/v) medium according to Ibrahim (2006).

4- Soil infestation with M. phaseolina:

Inocula of *M. phaseolina* were mixed thoroughly with soil of each pot at the rate of 2% w/w and covered with a thin layer of sterilized soil. Infested pots were irrigated and kept for 7 days until sowing.

5- Seed treatments:

5. a. *Microwaves treatments:*

Non-sterilized sunflower seeds (cv.Giza111) were exposed to thermotherapy in a microwave oven (LG Electronics Inc. MS5642G, 1000 W, 2450 MHz) for 15, 30 sec, 1, 2 and 4 min heat treatments, each alone. For a single treatment, 100 seeds were distributed evenly, in one layer, in 9 cm diam. sterile open glass dishes. Petri dishes were placed on the rotated microwave plate. A 500 ml beaker containing 200 ml distilled water was placed in the center of the plate as an energy sink in order to prevent seed damage (Reddy *et al.,* 2000). After treatment, seeds were left to cool down at 20–25°C on a laminar flow. Seeds were then bagged in sterile plastic bags until used.

5. b. *Hot water treatments:*

Non-sterilized sunflower seeds (cv. Giza111) were exposed to another heat treatment by soaking in hot water singly for five exposure periods; 30 sec., 1, 2, 4 and 8 min. One hundred seeds were used for each exposure time in five replicates. Replicates of all sets were placed individually in 250 ml conical flask containing 200 ml distilled hot water (80^{o}C) and all sets were kept in a water bath at 80^{o}C to maintain temperature constant.

After treatment, seeds were cooled down at 20-25°C on a laminar flow. Seeds were collected and bagged in sterile plastic bags until used.

5. c. *Fungicide treatments:*

Rizolex-T 50% WP (Tolclofos-methyl 20% + thiram 30%) was used in this study at the rate of 3g/kg seeds for comparison. The desired amount of fungicide was thoroughly mixed with sunflower seeds in plastic bags with Arabic gum solution (5 ml/kg seed) as sticker and shacked for 10 min to insure uniform coverage of seed with the tested compounds. Treated seeds were then allowed to dry for 24 h before sowing.

6- Disease assessment:

Disease assessment was made 15 and 45 days after planting for pre- and post-emergence damping-off, respectively. The percentage of charcoal rot was estimated, at harvest (90 days after sowing) and calculations were processed as following:

(A)

$$\%\text{ Pre- emergence} = \frac{\text{Number of non germinated seeds}}{\text{Number of planted seeds}} \times 100$$

$$\%\text{ Post- emergence} = \frac{\text{Number of dead seedlings}}{\text{Number of planted seeds}} \times 100$$

(B)

$$\%\text{ charcoal rot} = \frac{\text{Number of plants with root - rot}}{\text{Number of planted seeds}} \times 100$$

$$\%\text{ Healthy plants} = \frac{\text{Number of survived healthy plants}}{\text{Number of planted seeds}} \times 100$$

(C) Percentages of treatment efficacy in reducing disease infection were calculated as follows:

$$\%\text{ Treatment efficiency} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

$$\%\text{Treatment efficiency to fungicides efficacy} = \frac{\text{Treatment efficiency}}{\text{Fungicides efficiency}} \times 100$$

7- *Greenhouse experiments:*

Pot experiments were carried out during 2016 season at Agric. Res. Cent, Giza in order to study the effect of thermo seed treatments in controlling charcoal rot incidence (%). The treated sunflower seeds were sown in 50 cm-diameter pots containing sterilized soil previously infested or not with *M. phaseolina* (2% w/w). Ten seeds were sown per pot; five replicates (pots) were used for each treatment. Disease assessment was recorded as a percentage of pre- and post-emergence along with the percentage of charcoal rot incidence.

8- *Field experiments:*

Field experiments were performed at Ismailia governorate during 2017 and 2018 growing seasons to study the impact of thermo seed treatments in controlling charcoal rot incidence. The selected fields were known to have disease history with charcoal-rot pathogen. Complete randomized blocks design was followed, where four replicates of each treatment representing one experimental unit. Ninety seeds were used in each replicate which included three rows 3 m in length and 50 cm width with 10 cm spacing between halls (one seed/hill). The treated sunflower seeds were sown on the first week of April. Cultural practices and fertilization for sunflower crop were applied as recommended. Disease assessment was recorded as mentioned before.

9- *Statistical analysis:*

Comparison between means was performed using LSD at $p < 0.05$ and the standard error was calculated using the Statistical analysis software "COSt at 6.4" (Anon., 2005).

Results

1- *Occurrence (%) of sunflower seed-borne fungi:*

Various fungi were isolated from different samples of sunflower seed, representing different growing locations in Ismailia and Beni-Suef governorates (Table 1). Fifteen fungal species belonging to 10 genera were isolated from sunflower seeds collected from Beni-Suef fields and thirteen fungal species belonging to 8 genera were isolated from sunflower seeds collected from Ismailia fields by using standard blotter method.

Generally, surface sterilized seeds yielded less population of seed-borne fungi in comparison to non-surface sterilized seeds. *Alternaria alternata* was the most predominantly isolated fungus from sunflower seeds collected from the two locations and recorded the highest frequency followed by *Aspergillus flavus* and *A. niger*, while both of *Cladosporium* sp. and *Rhizoctonia solani* were occurred in low frequencies in Beni-Suef samples (Table, 1).

Macrophomina phaseolina was the main pathogen isolated from sunflower seeds collected from the two locations followed by *Fusarium oxysporum* and *Fusarium* sp. *Rhizoctonia solani* occurred in low frequency from only Beni-Suef samples (Table, 1).

2- *Impact of some thermo-seed treatments on occurrence (%) of M. phaseolina and germination (%) of sunflower seeds*.

This experiment was carried out to evaluate the impact of microwave radiation at five exposing periods (15, 30 sec, 1, 2 and 4 min) and hot water treatment at five soaking periods (30 sec, 1, 2, 4 and 8 min.) on the occurrence (%) of *M. phaseolina* on sunflower seeds as well as seeds germination (Table, 2).

The results in Table (2) show that there was a high impact of thermo treatments on the occurrence of *M. phaseolina* on sunflower seeds as well as seeds germination. In this regard, the treatment of hot water gave higher effect on frequency of *M. phaseolina* than the microwave treatment. Increasing the exposure time of the microwave radiation or soaking in hot water led to increase their effects on occurrence of *M. phaseolina* in sunflower seeds with parallel effects on germination. Moreover, presented results also show that hot water treatment at the time of 2 min soaking was the best treatment in reducing *M. phaseolina* occurrence followed by exposure to microwave radiation treatment at the time of 1 min (Table, 2).

Table 1: Occurrence (%) of sunflower seed-borne fungi using blotter method

Fungi	Beni-Suef				Ismailia			
	NSS[1]	SD[2]	SS[3]	SD[2]	NS[1]	SD[2]	SS[3]	SD[2]
A. alternata	90	3.536	75	4.472	85	2.236	60	3.962
Alternaria spp.	15	2.739	10	2.739	10	2.739	0	0.000
A. flavus	80	5.701	75	5.701	80	4.183	60	4.472
A. niger	75	3.536	60	4.183	70	4.472	20	2.236
Aspergillus sp.	70	2.236	45	2.236	80	4.183	40	2.739
Cladosporium sp.	10	2.588	0	0.000	0	0.000	0	0.000
F. oxysporum	45	3.536	30	2.236	30	5.477	20	4.183
F. solani	10	1.789	10	2.739	20	2.588	10	0.000
Fusarium sp.	30	4.087	10	2.236	30	2.168	10	2.739
M. phaseolina	50	2.625	30	3.477	35	2.881	25	3.536
Penicillium sp.	70	4.183	60	4.472	70	4.775	60	2.739
R. solani	15	2.739	5	2.236	0	0.000	0	0.000
Rhizopus spp.	75	4.183	30	6.519	85	4.336	40	4.183
Stemphylium sp.	60	4.472	25	4.183	50	2.168	20	3.536
T. roseum	30	2.168	20	2.236	40	3.507	25	2.490

1= Non-surface sterilization, 2= Standard deviation, 3= Surface sterilization.

Table 2: Impact of some thermo-treatments on the occurrence (%) of *M. phaseolina* on sunflower seeds as well as germination (%)

Treatments	Time	Occurrence (%) of *M.phasiolina*		Germination (%)
		NSS[1]	SS[2]	
Microwave	15 sec	40[b]	25[b]	100[a]
	30 sec	30[bc]	15[c]	100[a]
	1 min	10[de]	10[cd]	90[b]
	2 min	0.0[e]	0.0[e]	0.0[c]
	4 min	0.0[e]	0.0[e]	0.0[c]
Hot water	30 sec	20[cd]	15[c]	100[a]
	1 min	10[de]	5[de]	100[a]
	2 min	5[e]	0.0[e]	90[b]
	4 min	0.0[e]	0.0e	0.0[c]
	8 min	0.0[e]	0.0[e]	0.0[c]
Control		55[a]	35[a]	100[a]

1= Non-sterilized seeds, 2= Sterilizes seeds.
Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

3- *Impact of some thermo-treatments on Pre- and Post-emergence damping-off and disease incidence of charcoal rot on sunflower crop under infested and non-infested soil with M. phaseolina:*

Effectiveness of exposure sunflower seeds to microwave radiation for three exposing times, each alone (15, 30 sec and 1 min) and hot water treatment at three soaking times (30 sec, 1, and 2 min.) on pre- and post-emergence damping-off. Sunflower survivals were also recorded at maturity under infested and non-infested soils with *M. phaseolina* are shown in Tables (3 and 4).

In general, disease incidence (at the different stages) was significantly decreased while the percentage of survivals at mature stage was increased by all tested treatments in comparison with the control. Data also show that all treatments gave higher effect with non-infested soil than infested soil by *M. phaseolina*. Moreover, there was positive relationship between exposure time of microwave radiation or soaking in hot water and their effects on disease incidence, which indicated clearly that soaking in hot water treatment effectively reduced disease incidence than microwave radiation. In this respect, soaking in hot water for 2 min was the most effective treatment for decreasing pre- and post-emergence damping-off, as well as increasing survival percentages of sunflower plants followed by soaking in hot water for 1 min then exposure to microwave radiation for 1 min (Tables 3 and 4).

Table 3: Impact of some thermo-treatments on pre- and post-emergence damping-off and disease incidence of charcoal rot on sunflower crop under non-infested soil with *M. phaseolina*, greenhouse experiment

Treatments	Time	Damping-off		Total	Charcoal-rot	Plant survival
		Pre-	Post-			
Microwave	15 sec	8.0^{b}	10.5^{b}	18.5^{b}	11.0^{b}	70.5^{d}
	30 sec	3.0^{d}	8.0^{c}	11.0^{c}	11.0^{b}	78.0^{c}
	1 min	3.0^{d}	3.0^{e}	6.0d^{e}	6.0^{c}	88.0^{b}
Hot water	30 sec	5.5^{c}	10.5^{b}	16.0^{b}	6.0^{c}	78.0^{c}
	1 min	3.0^{d}	5.5^{d}	8.5^{cd}	3.5^{d}	88.0^{b}
	2 min	0.5^{e}	3.0e	3.5^{e}	1.0^{e}	95.5^{a}
Control		10.0^{a}	12.0^{a}	22.0^{a}	13.0^{a}	65.0^{e}

Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

Table 4: Impact of some thermo-treatments on pre- and post-emergence damping-off and disease incidence of charcoal-rot on sunflower crop under infested soil by *M. phaseolina*, greenhouse experiment

Treatments	Time	Damping-off		Total	Charcoal-rot	Plant survival
		Pre-	Post-			
Microwave	15 sec	16.5^{b}	16.5^{b}	33.0^{ab}	18.5^{a}	48.5^{c}
	30 sec	10.0^{d}	16.0^{b}	26.0^{abc}	12.5^{b}	61.5^{b}
	1 min	10.0^{d}	15.0^{b}	25.0^{abc}	10.0^{c}	65.0^{ab}
Hot water	30 sec	12.5^{c}	10.0^{c}	22.5^{bc}	19.0^{a}	58.5^{b}
	1 min	10.0^{d}	10.0^{c}	20.0^{c}	12.5^{b}	67.5^{ab}
	2 min	10.0^{d}	10.0^{c}	20.0^{c}	10.0^{c}	70.0^{a}
Control		18.0^{a}	20.0^{a}	38.0^{a}	20.0^{a}	42.0^{c}

Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

4- Impact of some thermo-treatments on pre- and post-emergence damping-off and disease incidence of charcoal rot on sunflower crop under field conditions during 2017 and 2018 growing seasons:

Data in Tables (5 and 6) indicate that all thermo-treatments at different times of exposure to microwave radiation, soaking in hot water or fungicide (Rizolex-T 50% WP) showed significant reduction of pre- and post-emergence damping-off and charcoal rot diseases compared to non-treated control in the two successive season (2017 and 2018).

Data of field experiments confirmed that hot water treatment gave better effect on disease incidence than microwave radiation treatment. Increasing time of seed exposure to microwave radiation or seed soaking in hot water led to decrease of disease incidence as well as increase of survival percentages.

Data in Tables (5 and 6) show also that soaking in hot water for 2 min was the best treatment for decreasing all disease incidences and increasing plant survival percentages followed by soaking in hot water for 1 min and exposure to radiation microwave for 1 min.

Table 5: Impact of some thermo-treatments on pre- and post-emergence damping-off and disease incidence of charcoal-rot on sunflower crop under field conditions during season 2017

Treatments	Time	Damping-off		Total	Charcoal-rot	Plant survival
		Pre-	Post-			
Microwave	5 sec	14.50^{ab}	12.00^{b}	26.50^{b}	16.50^{ab}	57.00^{d}
	30 sec	12.00^{bc}	8.00^{c}	20.00c	15.25^{bc}	64.75^{c}
	1 min	10.00^{c}	3.25^{de}	13.25^{de}	13.00de	73.75^{b}
Hot water	30 sec	11.00^{c}	4.75^{d}	15.75^{d}	16.25^{ab}	68.00c
	1 min	7.00^{d}	2.75^{de}	9.75^{ef}	13.75^{cd}	76.50^{b}
	2 min	5.00^{d}	1.50^{e}	6.50f^{g}	11.50^{ef}	82.00^{a}
Rizolex-T 50% WP		2.00^{e}	1.25^{e}	3.25^{g}	11.00^{f}	85.75^{a}
Control (untreated)		16.00^{a}	14.50^{a}	30.50^{a}	18.00^{a}	51.50^{e}

Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

Table 6: Impact of some thermo-treatments on pre- and post-emergence damping-off and disease incidence of charcoal rot on sunflower crop under field conditions during season 2018

Treatments	Time	Damping-off		Total	Charcoal-rot	Plant survival
		Pre-	Post-			
Microwave	5 sec	14.00^{b}	9.00^{b}	23.00^{b}	15.00^{b}	62.00^{e}
	30 sec	11.00^{c}	6.00^{c}	17.00^{c}	12.50^{cd}	70.50^{d}
	1 min	8.00^{de}	1.50^{e}	9.50^{e}	10.00^{e}	80.50^{c}
Hot water	30 sec	10.00^{cd}	4.00^{d}	14.00^{d}	14.00^{bc}	72.00^{d}
	1 min	6.75^{e}	2.25^{e}	9.00^{e}	11.00^{de}	80.00^{c}
	2 min	4.00^{f}	1.50^{e}	5.50^{f}	7.500^{f}	87.00^{b}
Rizolex-T 50% WP		2.75^{f}	0.75^{e}	3.50^{f}	6.00^{f}	90.50^{a}
Control (untreated)		17.50^{a}	11.50^{a}	29.00^{a}	17.00^{a}	54.00^{f}

Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

Data in Table (7) show that some thermo-treatments gave efficiency in reducing damping-off and sunflower charcoal rot diseases approximately close to the fungicide efficiency. In this respect, seed soaking in hot water for 2 min was the closest one to the fungicide efficiency in reducing sunflower pre- and post-emergence damping-off and charcoal rot followed by soaking in hot water for 1 min and exposure to radiation of microwave for 1 min. compared to other treatments during 2017 and 2018 growing seasons.

Table 7: Percentage efficiency to fungicide efficiency of different treatments on damping-off and charcoal rot of sunflower grown under field conditions during two seasons 2017 and 2018.

Seasons	Treatment	Time	Damping-off		Charcoal rot %	Plant Survival %
			Pre	Post		
2017	Microwave	5 sec	10.71	18.87	21.43	16.06
		30 sec	28.57	49.06	39.28	38.69
		1 min	42.86	84.91	71.43	64.97
	Hot water	30 sec	35.71	73.58	25.00	48.18
		1 min	64.29	88.68	60.71	73.00
		2 min	78.57	98.11	92.85	89.06
	Rizolex-T 50% WP		100.00	100.00	100.00	100.00
2018	Microwave	5 sec	23.73	23.26	18.18	21.92
		30 sec	44.07	51.16	40.91	45.21
		1 min	64.40	93.02	63.63	72.61
	Hot water	30 sec	50.84	69.77	27.27	49.32
		1 min	72.88	86.04	54.54	71.24
		2 min	91.52	93.02	86.36	90.41
	Rizolex-T 50% WP		100.00	100.00	100.00	100.00

* The data was calculated based on the data in Tables 5 and 6

** % Treatments efficiency in reducing the diseases infection was calculated as follows: [(control-treatment)/control)] x 100

***% Treatment efficiency to fungicides efficacy was calculated as follows: (Treatment efficacy / fungicides efficacy) x 100

5- *Impact of some thermo-treatments on total seed yield of sunflower under field conditions during the two seasons, 2017 and 2018.*

Data in Table (8) illustrate that sunflower seed yield production was significantly varied among the tested thermo-treatments during the two seasons, 2017 and 2018. In this respect, the highest total seed yield in the two seasons, except fungicide treatment, was produced with soaking in hot water for 2 min followed by soaking in hot water for 1 min and exposure to radiation microwave for 1 min. Data also indicated that there were positive relationships between increasing the time of soaking or exposure and their effect on total sunflower seed yield (Table, 8).

Table 8: Impact of some thermo-treatments on total seed yield of sunflower under field conditions during two growing seasons, 2017 and 2018.

Treatment	Time	Season 2017		Season 2018	
		Seed yield (kg/plot)	z) Increase (%)	Seed yield (kg/plot)	z) Increase (%)
Microwave	5 sec	1.94^{d} (y	11.49	2.18^{e}	13.54
	30 sec	2.22^{c}	27.59	2.25^{de}	17.19
	1 min	2.37^{abc}	36.21	2.50^{bc}	30.21
Hot water	30 sec	2.26^{bc}	29.89	2.38^{cd}	23.96
	1 min	2.44^{abc}	40.23	2.50^{bc}	30.21
	2 min	2.49^{ab}	43.10	2.60^{b}	35.42
Rizolex-T 50% WP		2.60^{a}	49.43	2.75^{a}	43.23
Control		1.74^{d}		1.92^{f}	

y) Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05). z) Increase related to the control

Discussion

Macrophomina phaseolina Tassi (Goid) is considered one of the most destructive soil- and seed-borne fungi affecting sunflower in Egypt and all over the world including Egypt causing losses in total seed yields and oil production (Gulya, 2002; Aboshosha *et al.,* 2007 and Ghonim *et al.,* 2014).

Various fungi were isolated from samples of sunflower seed collected from Ismailia and Beni-Suef governorates. Fifteen and 13 fungal species belonging to 10 and 8 genera were isolated from Beni-Suef and Ismailia fields using the standard blotter method, respectively. Generally, surface sterilized seeds yielded less population of seed-borne fungi in comparison to non-sterilized seeds. Similar results were obtained by Afzal *et al.* (2006) and Ghonim *et al.* (2014) who stated that chloral disinfection of sunflower seeds effectively reduced the microbial contamination. *Alternaria alternata* was the most predominantly isolated fungus from sunflower seeds obtained from the two locations investigated and recorded the highest frequency which could be the cause of sunflower leaf spot, while, *Macrophomina phaseolina* was the main seed-borne fungus isolated from sunflower seed in the two locations followed by *Fusarium oxysporum* and *Fusarium* sp. In this respect, current results were found to be in a harmony with those obtained by Afzal *et al.* (2006); Ghonim *et al.* (2014) and Khalil *et al.* (2014).

Results of this study also proved that there was a high impact of thermotherapy on the occurrence of seed-borne fungus *M. phaseolina* as well as seeds germination. Moreover, increase the exposure time of the microwave radiation or soaking in hot water treatment led to increase their effects on occurrence of *M. phaseolina* in sunflower seeds. These results are in agreement with those obtained by Forsberg, (2004) and Jahn *et al.* (2006) who demonstrated that various thermotherapy treatments can be effective in reducing or eliminating seed-borne pathogens. However, adverse effects on germination of sunflower seeds as a result of increasing time of exposure to microwave radiation or soaking in hot water may be due to the disadvantages associated with this treatment, such as injury to the seed itself through damage to the seed coat or the effect on seed embryo bioactivity (El-Abbasi, 2004 and Allison, 2014). Data also showed that treatment of hot water gave higher effect on frequency of *M. phaseolina* than radiation microwave treatment that can be interpreted as humid temperature may penetrate plant tissues and the outer layers of the seed and give a high effect on seed-borne fungi. On the other hand, clarifying the difference between the common hot water treatment and the microwave heating is of great importance. In the first type, heat current can transmit from the outer surface of a seed to its inner layers, while microwave heat begins from the inner to the outer layers because the waves themselves initiate very fast movement of atoms and particles which generate heat (Kraus and Fleisch, 1999).

Results of greenhouse and field experiments clearly showed that exposure of sunflower seeds to microwave radiation for 15, 30 sec and 1 min and also soaking in hot water for 30 sec, 1, and 2 min led to significant decrease of the incidence of pre- and post-emergence damping-off as well as charcoal rot diseases with subsequent increase of sunflower seed yield. Moreover, there is a positive relationship between time of exposure to microwave radiation, soaking in hot water and treatment effect on disease incidence which clearly proved that efficacy of soaking in hot water treatment reduced disease incidence effectively than the efficacy of exposure to microwave radiation. In this respect, many workers stated that seed treatment with hot water reduced seed-borne pathogens fungi; bacteria and even viruses of different crops (Nega *et al.,* 2003; Muniz, 2004 and Rahman *et al.,* 2008), their findings supported the results of the present study. They suggested a possible phenomenon of decreasing the seed-borne pathogen by hot water treatment where, firstly the temperature acted upon the fungal contaminants and with increasing the temperature it penetrates seed layers and kills pathogen embedded deeper and deeper in the seeds. Though hot water seed treatment reduced seed-borne infection but in certain cases decrease germination percentages of the seeds. On the other hand, the effect of microwave radiation due to the fact that it produces energy through dielectric heating, which is a process that utilizes high-frequency electromagnetic radiation to heat dielectric materials through the aligning of dipole water molecules. When dielectric materials, such as seed, are exposed to microwave radiation the realignment of the water molecules creates friction, which produces heat and increases seed temperatures (Bouraoui *et al.,* 1993). Increased exposure time gives an effect on percentage of seed germination which decreases rapidly as a result of increased temperature (Knox *et al.,* 2013). Allison (2014) stated that, microwave exposure impact on pathogen colonization in the seed. A linear reduction in visible pathogen growth was observed and with every second of microwave exposure, pathogen colonization of the seed decreased 0.14% and 0.10% for navy and pinto bean, respectively. The reductions in colonization in combination with the slight decrease in seed germination between 30-50 sec. of exposure were indicative of the potential of microwave radiation as an alternative seed treatment option.

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تأثير المعالجة الحرارية لبذور عباد الشمس على مقاومة الفطر *Macrophomina phaseolina* المسبب لمرض العفن الفحمي

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تم عزل عدد من الفطريات المختلفة من عينات بذور عباد الشمس التي تم جمعها من حقلين في كل من محافظتي الإسماعيلية وبني سويف. حيث تم عزل خمسة عشر نوعًا من الفطريات التي تنتمي إلى ١٠ أجناس من حقل فى بني سويف وثلاثة عشر نوعًا من الفطريات التي تنتمي إلى ٨ أجناس من حقل فى الإسماعيلية. سجل فطر *Alternaria alternata* أعلى نسبة تواجد في بذور عباد الشمس التي تم جمعها من الموقعين تلاه الفطر *Aspergillus flavus* ثم الفطر *A. niger*. كان الفطر *Macrophomina phaseolina* هو أكثر الفطريات الممرضة تواجدا في بذور عباد الشمس في الموقعين، تلاه الفطر *Fusarium oxysporum* والفطر *Fusarium* sp. أعطي تطبيق المعالجة الحرارية لبذور عباد الشمس عن طريق التعرض لأشعة الميكروويف بإستخدام خمس فترات متفاوتة (١٥و ٣٠ ثانية و ١ و ٢ و ٤ دقائق) والنقع في المياه الساخنة بإستخدام خمس فترات نقع (٣٠ ثانية و ١ و ٢ و ٤ و ٨ دقائق) تأثيراً معنوياًعلى تواجد الفطر *M. phaseolina* كأحد الفطريات المنقولة بالبذرة وكذلك نسبة إنبات بذور عباد الشمس. علاوة على ذلك ، أدى زيادة وقت التعرض لإشعاع الميكروويف أو النقع في الماء الساخن إلى زيادة تأثيرها. كان للنقع في الماء الساخن أثراً سالباً على نسبة تواجد الفطر *M. phaseolina* عنه في حالة التعرض لأشعة الميكروويف ، وبشكل عام أظهرت النتائج التي تم الحصول عليها أن استخدام اشعة الميكروويف والنقع في الماء الساخن قللت بشكل معنوي نسبة موت البادرات قبل وبعد الإنبات وكذلك الإصابة بمرض العفن الفحمي سواء كانت الزراعة في التربة الغير معدية أو المعدية بالفطر *M. phaseolina* وكذلك في التجارب الحقلية كما أدت إلى زيادة محصول بذور عباد الشمس. وفي هذا الشأن فإن أكبر تأثير تم الحصول عليه عندما تم نقع بذور عباد الشمس في الماء الساخن لمدة ٢ دقيقة تلاها كل من النقع في الماء الساخن لمدة ١ دقيقة والتعرض لأشعة الميكروويف لمدة ١ دقيقة. كما أثبتت النتائج أيضًا أن بعض العلاجات الحرارية أعطت كفاءة في تقليل الإصابة بمرض العفن الفحمي في عباد الشمس تقترب من كفاءة المبيد الفطري المختبر(ريزولكس T-50) خلال موسمي ٢٠١٧ ،و ٢٠١٨ وفي هذا الصدد كانت معاملة النقع في الماء الساخن لمدة دقيقتين هي الأقرب.