Zagazig J. Agric. Res., Vol. 50 No. (1) 2023 67-80

Plant Protection and Pathology Research

Available online at http://zjar.journals.ekb.eg
http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

IDENTIFICATION AND CONTROL OF PATHOGENIC RACES OF *Ustilago nuda* LOOSE SMUT CAUSAL PATHOGEN OF BARLEY

Badawy R.H. Mohdly[1]*, T.E. Abd El-Baset[1], M.M. Abd El-Ati[2], Heba G.A. Gomaa[2], Sally E.A. El-Wakeel[2] and K.A. Amer[2]

1. Plant Pathol. Res. Inst., Agric. Res. Cent., Giza, Egypt

2. Field Crops Res. Inst., Agric. Res. Cent., Giza, Egypt

Received: 03/10/2022 ; Accepted: 18/12/2022

ABSTRACT: Identification of *Ustilago nuda* (Jens.) Rostr., the causal agent of barley (*Hordeum vulgare* L.) loose smut disease using the Random Amplified Polymorphic (DNA) markers is faster and easier than using of the traditional methods (differential cultivars),where identify physio-logical races may needs several years. Also, at the two locations, Giza and Sakha Agricultural Research Stations, several effectors were tested to find out their effectiveness in ridding the grains of the fungus that resides inside them, *i.e.* (soaking in some natural oils, exposure to Gamma ray and dressing with some new fungicides). Between nine effectors that were tested, two only (Raxil and Simasol) proved to have the highest degree of efficacy (up to 100%), Hattric and pine oil were the most effective treatments (98.4 and 97.9%, respectively). Gamma radiation at 250 Gy followed by clove oil and castor oil changed reactions to moderately resistant against *U. nuda*. Infection of barley seed caused significant differences among all studied plant traits.

Key word: Barley, Loose smut, *Ustilago nuda*, Fungicides, Gamma ray, Natural oils, Dry and wet, inoculation methods.

INTRODUCTION

Cultivated barley (*Hordeum vulgare* spp. *vulgare* L.) is an economically important temperate cereal. It is well adapted to diverse environmental stresses, including low rainfall and cold winter temperatures **(Van Oosterom and Acevedo, 1992),** barley is primarily grown as feed grain and grain for malting, but its importance in human consumption is also growing **(Baik and Ullrich, 2008).**

Loose smut fungus colonize the seed without causing obvious disease symptoms before heading. The mycelium becomes dormant in mature seeds, but when infected seed germinates at the following spring, the mycelium begins to grow and penetrates the growing point **(Ramdani *et al*., 2004)**.

Loose smut is transmitted mainly from year to year by infected seeds**.** It can also spread through the air from infected plants to healthy plants during the growing season, but not over long distances **(Wunderle *et al*., 2012)**.

The disease can cause significant losses in grain yield (up to 5 – 7%) and reduction of resulting flour from barley and wheat. Smut and rust were the main concern of farmers until the 20th century in most of the barley and wheat growing areas **(Agrios, 2005)**, barley yield are reduced according to the percent of smutted heads, since most infected heads produce no seed, the amount of loose smut varies from year to year depending on environmental conditions during flowering, cool humid weather accompanied by high showers or heavy dews are most favorable for infection. The disease is usually seen at wet and cool higher elevations **(Saari *et al*., 1996)**.

*** Corresponding author: Tel. :**+201119767400
E-mail address: bm01119767400@gmail.com

Infection of barley plants takes place shortly before the flowering period, the maximum infection occurs during flowering and during two days after the flowering period. Wind, rain, insects, and other agents carry the olive black teliospores from a smutted head to the open flowers of a healthy head. Here, in the presence of moisture, with a temperature of 60 to 71 F (16 – 22^{o}C). The spores quickly germinate and grow down the female parts of the flower to invade the young embryo **(Malik and Batts, 1960)**. Infection also may occur by direct penetration of the embryo wall, but within a week after flowering, the ovary and flower parts become resistant to infection **(Nielsen, 1987).**

The main objective of this study is collecting loose smut isolates from different Egyptian locations and identification of *Ustilago nuda* races of barley, as well as induce resistance to loose smut disease using natural, physical and chemical seed treatments.

MATERIALS AND METHODS

Molecular Identification

Genomic DNA was extracted from fungal samples using a DNA easy Tissue extraction kit (Qiagen, CA, USA) according to the manufacturer's instructions. ITS rRNA analysis **(Nelson *et al*., 2011)**.

PCR Reactions

The PCR amplification was performed in a total volume of 50 ul, containing 1X reaction buffer, 1.5 mM $MgCl_2$, 1U *Taq* DNA polymerase (promega), 2.5mM dNTPs, 30 poml of each primer and 30 ng genomic DNA (Table 1) **(White *et al*., 1990)**.

Thermo-cycling PCR program.

Polymerase Chain Reaction (PCR) amplification was performed in a Perkin-Elmer/ GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 30 sec., an annealing step at 45ºC for 30 sec. and an elongation step at 72ºC for 1 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

Detection of the PCR products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 100bp DNA ladder was used as a molecular size standard. Products of PCR were visualized on UV light and photographed using a Gel Documentation System (**BIO-RAD, 2000**).

Purification of PCR products

Amplified products for all PCR were purified using EZ-10 spin column PCR products purification. Reaction mixture PCR was transferred to 1.5 ml microfuge tube and three volumes was added of binding buffer 1 after that the mixture solution was transferred to the EZ-10 column and let it stand at room temperature for 2 minutes then centrifuge. Seven hundred and fifty ul of wash solution was added to the column and centrifugal at 10.000 rpm for two minutes, washing was repeated, and 10.000 rpm was spine for an additional minute to remove any residual wash solution. The column was transferred into a clean 1.5 ml microfuge tube and 50 ul adds of elution buffer, incubated at room temperature for 2 minutes and purified DNA at -20°C was stored **(Brown, 1996**).

ITS Sequencing Analysis

The sequencing of the product PCR was carried through in an automatic sequencer ABI PRISM 3730XL Analyzer using Big Dye TM Terminator Cycle Sequencing Kits following the protocols supplied by the manufacturer, single-pass sequencing was performed on each template using Rbcl Forward primer. The fluorescent-labeled fragments were purified from the unincorporated terminators using an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Microgen Company).

Computational Analysis (BLASTn) ITS

The sequences were analyzed using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned using Align Sequences Nucleotide BLAST.

Table 1. Primer ITS Forward and Reverse, Sequence and product size

Primer Code	Sequence	Product Size
(ITS-1) F	5′- TCCGTAGGTGAACCTGCGG -3′	600bp
(ITS-4) R	5′- TCCTCCGCTTATTGATATGC-3′	

Field experiments

Two randomized complete block trials were conducted at two Egyptian locations, Giza and Sakha Agricultural Research Stations, Agricultural Research Center (ARC) Egypt, on barley variety Giza 123. Thirty six lines were sown with three replications in research farm area, each four line was sown in a single plot 2x3 m in size with twenty infected seeds in each line as well as the control, during November of 2020/2021 and 2021/2022 growing seasons to evaluate the effect of nine effectors on disease incidence and some plant parameters.

The disease is easily recognized at the time of heading by the characteristic duty black appearance of diseased heads, the infected heads emerge from the boot one to three days earlier of those healthy plants. The infected heads are collected for used in the following year to carry out the experiment. This a us done in the first season of the experiment ,the line were planted in 2 (cm) long row, and five ears of each entry were inoculated at growth stage 59 days of **Zadoks *et al*. (1974)** and the scale of **Gupta *et al*. (1991).**

There are two methods of inoculation; dry method and wet method were conducted:

Dry method

For inoculation, dry spores of loos smut are introduced into the florets using: (a) forceps, (b) small brush, (c) dusting spores over entire spikes, after removing the top of centeral floret of each spikelet and clip the glumes of the two remaining florets to expose the stigma and anthers, use a smutted spike to dust teliospores onto clipped spike, then cover the spike with a paper bag. This is the method that used in experiments of **Cherewick and Cunninghom (1956), Joshi *et al.* (1988), Pandey and Gautam (1988)** and **Sammonr *et al.* (2015).**

Wet method

After removing the top of centeral floret of each spikelet and clip to expose the stigma and anthers, the spikelets are injected by a syringe with a suspension of *Ustilago nuda* teliospores in 0.01% Tween 20 (25mg of spores/100 ml of water) according to **Walters and Murray (1992)** and **Menzies *et al*. (2009).**

The infected grain will appear early, when it germinates at the following season, a dark mass of spores will form instead of healthy grain. Thus, pure isolates are obtained and used to identify races of (*Ustilago nuda*) by extracting Deoxynucleic acid (DNA) from 0.50 mg teliospores.

On a field-wide basis, the amount of yield loss is proportional to the percentage of infected heads during crop season. The seeds obtained of previous year were planted during the next season before the fungus grows up with the plant, these seeds were treated with systemic fungicides 24 h before planting, some other were treated with natural oils and the third other were exposed to Gamma rays.

The effectors used in these experiments:

The infected barley grains were treated before seeded by 24 h with:

a- Fungicides: Raxil (Tebuconazole) (1.2 ml), Hattric (Diniconazole) (0.7 ml) and Simasol (Nonyl phenol ethoxylate) (5.0 ml) Kg seeds, as a seed dressing treatment.

b- Natural oils: Clove oil (5.0 ml), pine oil (5.0 ml), and castor oil (5.0 ml), with 0.01% Tween 20, as a seed soaking treatment for 24 hours according to **Sallam *et al*. (2001)**.

c- Gamma ray (150, 200 and 250 Gy/1 minute) as a irradiation treatment. Irradiation was achieved at the National Center for Research

and Radiation Technology, Atomic Energy Authority, Nasr City, Egypt.

The loose smut infection (%) was calculated by counting the diseased and healthy ears on tiller basis **(Poehlman, 1945)**.

$$\text{Disease incidence} = \frac{\text{Number of infected}}{\text{Total number of the tested plant}} \times 100$$

Efficacy of the various treatments were determined at maturity by counting the smutted spikes as a percentage of the total number of the infected spikes according to the formula adopted by **Rewal and Jhooty (1985)** in which:

$$\text{Efficacy (\%)} = \frac{\text{\% infection in the control -\% infection in the treatment}}{\text{\% infection in the control}} \times 100$$

To evaluate the effect of the nine effectors on some plant parameters of barley plants inoculated with loose smut fungus under field conditions, grain yield per plot (Ardab /Feddan), plant high (cm), spike grains weight (g), number of grains /spike and 1000-grain weight (g), were estimated during and after harvesting. Data were statistically analyzed according to **Snedecor (1957)**.

RESULTS

Monitoring and Identification of Collected *Ustilago nuda* Races and Purified from Different Locations in Egypt

Polymerase Chain Reaction PCR ITS amplification

Polymerase Chain Reaction (PCR) products of approximately 600bp amplified with the ITS-1 F and ITS-4R primers and corresponding to the ribosomal RNA gene were obtained from two isolates (Fig. 1).

After purification of PCR products and sequencing, the BLAST-n alignments results showed that two sequences were associated with high levels of sequence similarity with the ribosomal RNA gene sequences for the *Ustilago nuda*. Sequences were deposited in the GenBank database of two accessions number *i.e*., OK576918 and OK576919.

Field Experiments

Effect of some effectors on disease incidence (%)

Disease incidence was recorded for each tested treatment, using the data obtained from the plant infected scores (infected spike) at certain time from appearance.

Data presented in Table 4 and Fig. 4, for Giza station during 2020/2021 and 2021/2022, growth seasons revealed that Raxil 2.5% and Simasol were the most effective treatments, resulting the lowest disease incidence exhibited high resistance with (0%) mean percentage of smut infected spikes, followed by the Hattric and pine oil resulting low levels of disease incidence. Loose smut indicate (not up to 2%) while clove oil and Gamma radiation (250 Gy) were not up to 11%. However treatments of castor oil and tow levels of Gamma radiation (200 Gy, 150 Gy) were found to be moderately resistant with (12.7, 14.25, 19.4%) smutted spikes, respectively. On the other hand, it was found that untreated control was remained highly susceptible with 82.75% smutted spikes.

Data presented in Table 5 and Fig. 5 for the second location (Sakha) during the two growing seasons (2020/2021 and 2021/2022), showed similar approximately results through the use of Raxil and Simasol where the mean percentage of smut infected spikes (0%). Also, the incidence of infection after use Hattric and pine oil application was not up to 2.5% and was moderately resistant with using of clove oil (6.1% disease incidence) followed by Gamma radiation 250 Gy (7.6%). With using of Gamma radiation at 150 Gy and 200 Gy highly disease incidence average of infected spikes (between 10% to 20%). On the other hand, the disease incidence was highly significant with the untreated control that averaged 84.5% smutted spikes.

The effectiveness of the effectors was estimated as the number of non- infected plants to the total number of tested plants for each treatment in the present study (Table 6) and (Fig. 6). The obtained data indicate that between the nine effectors that were tested against loose smut disease, two only proved to have the highest degree of efficacy (up to 100%) against the tested infected seed, Raxil and Simasol at the two investigated locations, Giza and Sakha Agricultural Research Stations, Hattric (98.4%) and pine oil (97.9%),

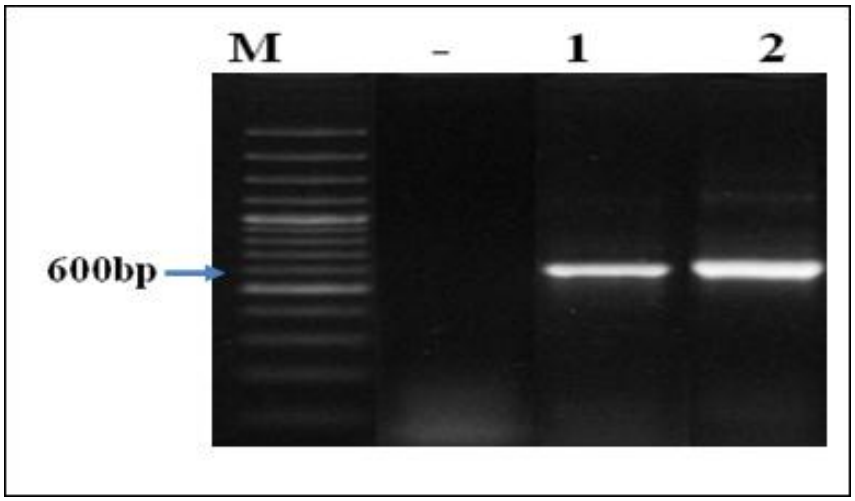

Fig. 1. ITS rRNA gene (~600 bp) of the two *Ustilago nuda* isolates amplified with universal primers. The amplicon was electroph-oretically resolved on a 1.5% agarose gel in 1X TBE buffer Lane1: 100bp DNA plus ladder

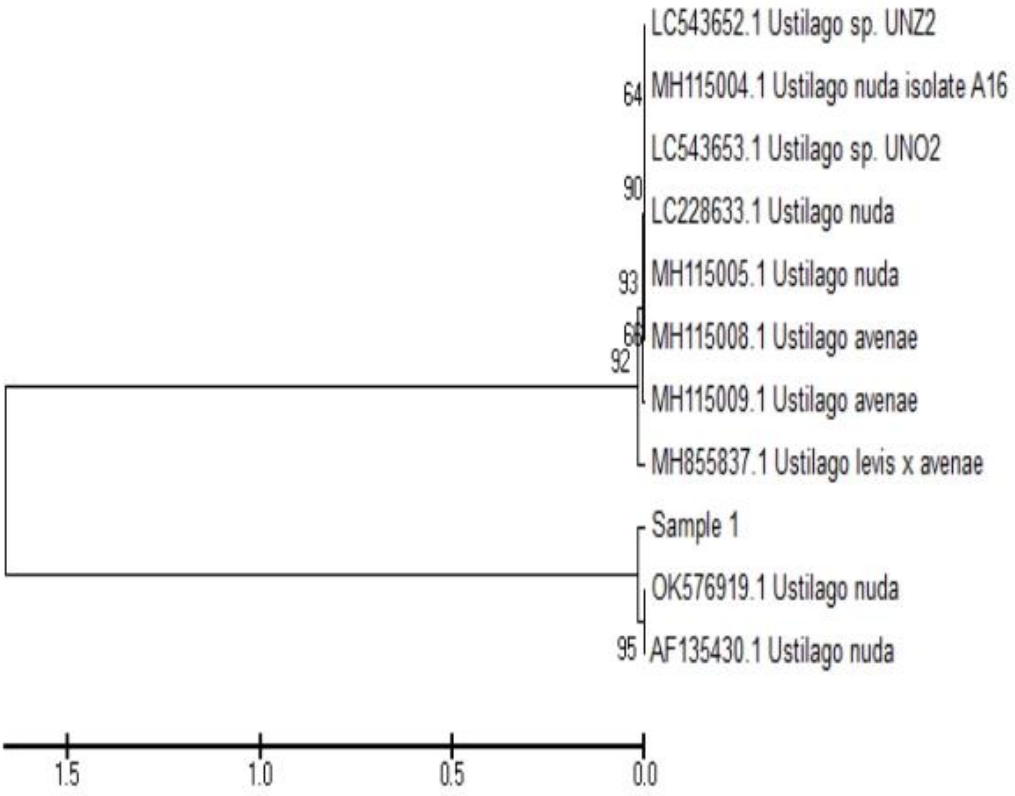

Fig. 2. Phylogenetic tree using (MEGA 5) of Sample 1 (OK576918) using ITS rRNA, showing names of fungi species and accession numbers

Table 2. ITS rRNA of sample 1 (OK576918) related fungi with similarity percentage of more than 99.56%, downloaded from GenBank database

Ustilago species	Accession No.	E-value	Query coverage (%)	Similarity (%)
U. sp.	LC543653.1	0.0	99	99.56
U. sp.	LC543652.1	0.0	99	99.56
U. nuda	OK576919.1	0.0	99	99.56
U. nuda	AF135430.1	0.0	99	99.56
U. nuda	MH115005.1	0.0	99	99.41
U. avenae	MH115008.1	0.0	99	99.41
U. avenae	MH115009.1	0.0	99	98.82
U. nuda	MH115004.1	0.0	99	98.38
U. nuda	LC228633.1	0.0	99	98.38
U. levis x avenae	MH855837.1	0.0	99	98.38

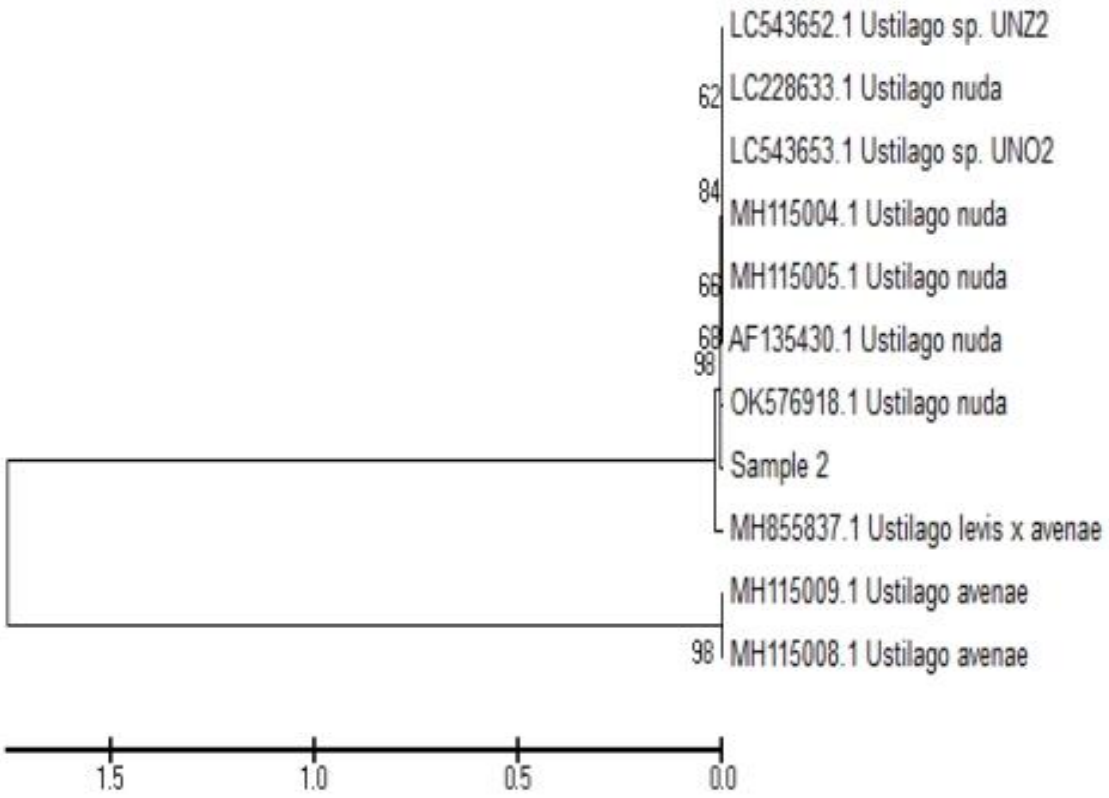

Fig. 3. Phylogenetic tree using (MEGA5) of Sample 2 (OK576919) using ITS rRNA, showing names of fungi species and accession numbers

Table 3. ITS rRNA of sample 2 (OK576919) related fungi with similarity percentage of more than 99.56%, downloaded from GenBank database

Ustilago species	Accession no.	E-value	Query coverage (%)	Similarity (%)
U. nuda	MH115005.1	0.0	99	99.41
U. nuda	AF135430.1	0.0	99	99.41
U. sp. *UNO2*	LC543653.1	0.0	99	99.26
U. sp. UNZ2	LC543652.1	0.0	99	99.26
U. nuda	MH115004.1	0.0	99	99.26
U. nuda	LC228633.1	0.0	99	99.26
U. nuda	OK576918.1	0.0	99	98.82
U. avenae	MH115009.1	0.0	99	98.38
U. avenae	MH115008.1	0.0	99	98.38
U. levis x avenae	MH855837.1	0.0	99	98.38

Table 4. Efficacy of barley seed treatments by nine effectors on loose smut disease under field conditions at Giza Research Station during 2020/21 and 2021/ 22 growing seasons

Effector	No. of tested plant						Disease incidence (%)			Treatment efficacy (%)		
	Infected			Non infected								
	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean
Hattric	06	00	03.0	159	165	162.0	3.6	0.0	1.8	95.8	100.0	97.9
Raxil	00	00	00.0	165	165	165.0	0.0	0.0	0.0	100.0	100.0	100.0
Simasol	00	00	00.0	165	165	165.0	0.0	00.0	0.0	100.0	100.0	100.0
Clove oil	16	10	13.0	149	155	157.0	09.7	06.1	07.9	88.5	92.4	90.5
Pine oil	05	00	02.5	160	165	162.5	03.0	00.0	01.5	96.5	100.0	98.3
Castor oil	24	18	21.0	141	147	144.0	14.5	10.9	12.7	83.1	86.3	84.7
G. ray (150Gy)	35	29	32.0	130	136	133.0	21.2	17.6	19.4	75.4	77.9	76.7
G. ray (200 Gy)	27	20	23.5	138	145	141.5	16.4	12.1	14.25	81.0	84.7	82.9
G. ray (250 Gy)	21	12	16.5	144	153	148.5	12.7	07.3	10.0	85.2	90.8	88.0
Control	142	131	136.5	023	034	28.5	86.1	79.4	82.75	00.0	00.0	00.0
L.S.D (5%)	-	-	-	-	-	-	-	-	-	12.11	10.78	-

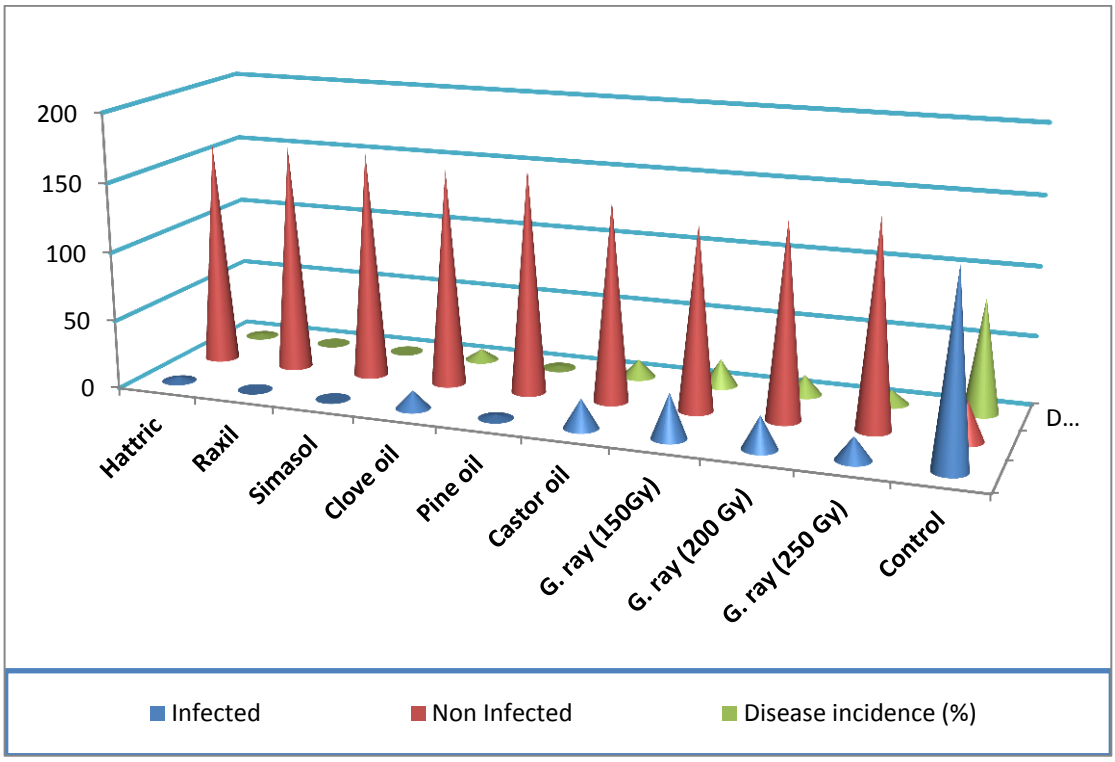

Fig. 4. Loose smut disease incidence (%) on barley plants under field conditions during 2020/21 and 2021/22 growing seasons at (Giza) Research Station

Table 5. Efficacy of barley seed treatments by nine effectors on loose smut disease under field conditions at Sakha Research Station during 2020/21 and 2021/ 22 growing seasons

Effector	No. of tested plant						Disease incidence (%)			Treatment efficacy (%)		
	Infected			Non infected								
	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean	20/22	21/22	Mean
Hattric	02	02	2.0	163	163	163.0	1.2	1.2	1.2	98.8	98.8	98.8
Raxil	00	00	0.0	165	165	165.0	0.0	0.0	0.0	100.0	100.0	100.0
Simasol	00	00	0.0	165	165	165.0	0.0	0.0	0.0	100.0	100.0	100.0
Clove oil	09	11	10.0	156	154	155.0	5.5	6.7	6.1	88.5	86.4	87.5
Pine oil	03	04	3.5	162	161	161.5	1.8	2.4	2.1	97.8	97.1	97.5
Castor oil	16	19	17.5	149	146	147.5	9.7	11.5	10.6	88.5	86.4	87.5
G. ray (150Gy)	30	32	31.0	135	133	134.5	18.2	19.4	18.8	78.4	77.1	77.8
G. ray (200 Gy)	17	22	19.5	148	143	145.5	10.3	13.3	11.8	87.8	84.3	86.1
G. ray (250 Gy)	12	13	12.5	153	152	152.5	7.3	7.9	7.6	91.4	90.7	91.2
Control	139	140	139.5	26	25	25.5	84.2	84.8	84.5	00.0	00.0	00.0
L.S.D (5%)	-	-	-	-	-	-	-	-	-	5.227	6.675	-

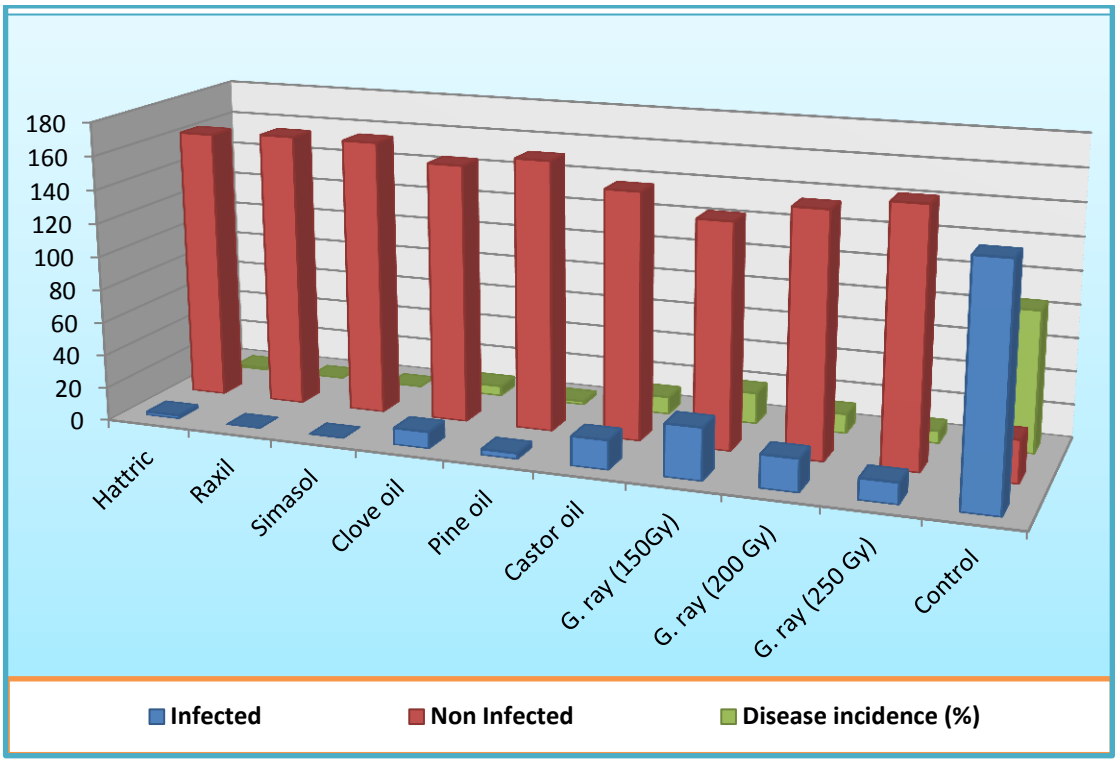

Fig. 5. Loos smut disease incidence (%) on barley plants under field conditions during 2020/21 and 2021/22 growing seasons at Sakha Research Station

Table 6. Efficacy of nine effectors against barley loose smut disease, under field conditions at Giza and Sakha Research Stations

Effector	Treatments efficacy (%)		
	Sakha	Giza	Mean
Hattric	98.8	97.9	98.4
Raxil	100.0	100.0	100.0
Simasol	100.0	100.0	100.0
Clove oil	90.5	87.5	89.0
Pine oil	98.3	97.5	97.9
Castor oil	87.5	84.7	86.1
G. ray (150Gy)	77.8	76.7	77.3
G. ray (200 Gy)	86.1	82.9	84.5
G. ray (250 Gy)	91.2	88.0	89.6
Control	00.0	00.0	-
L. S. D (5%)	6.3	7.1	-

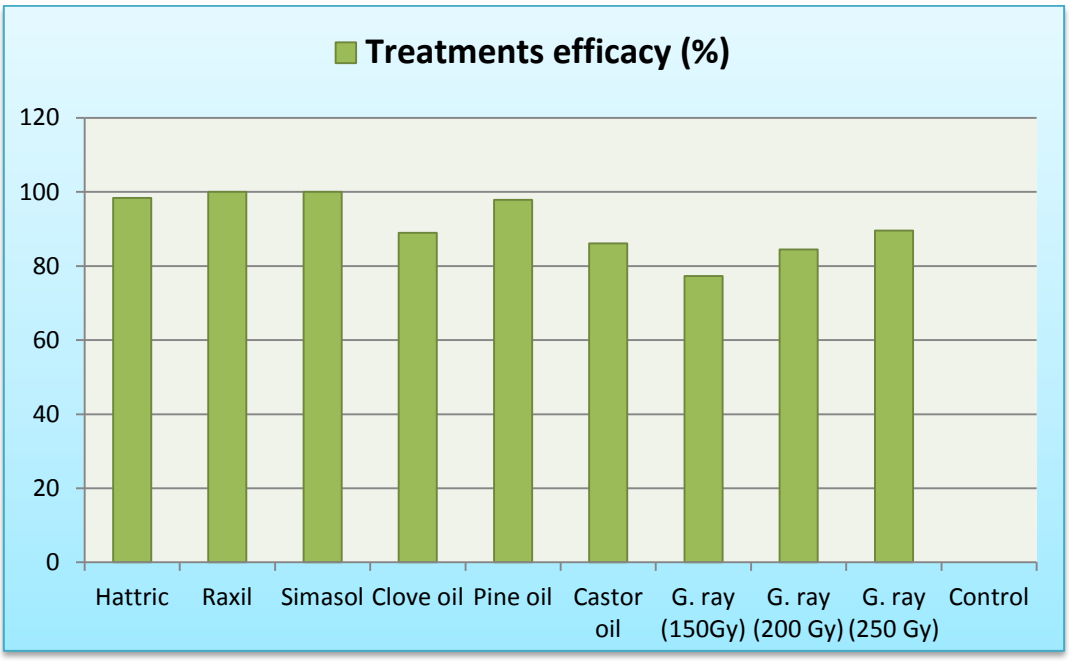

Fig. 6. Efficacy of some effectors against barley loose smut disease under field conditions at Giza and Sakha Research Stations during 2020/21 and 2021/22 growing seasons

were the most effective treatments, causing the lowest disease incidence of smut infected spikes. In addition, Gamma radiation 250 Gy (89.6%), clove oil (89.0%), and castor oil (86.1%) changed the efficacy to moderately resistant against *Ustilago nuda*.

Effect of some Effectors on Plant Parameters

Infection of barley seed showed significant differences among all investigate plant traits, *i.e.* plant height, number of grains/spike, spike grains weight, 1000 grain weight and grain yield (Table 7). For plant height, the highest values were obtained from pine oil and clove oil treatments) 105.7 and 103.1 cm at Giza and 109.8 and 105.2cm at Sakha, respectively), while the shortest plant were recorded for control treatment (81.6 at Giza and 84.5 cm at Sakha). Number of grains per spike, recorded the highest values for pine oil (60.53 grains at Giza and 62.73 grains at Sakha) compared to control treatment (41.67 grains at Giza and 40.65 grains at Sakha). Regarding grain weight (g) per spike, the most desirable values recorded for clove oil at Giza (2.82 g) and pine oil at Sakha (2.98 g), while the least values recorded for control treatment at Giza (1.93 g) and Sakha (2.11 g) locations. Concerning to 1000 grain weight, the highest values were obtained from pretreated seeds with pine oil (53.50 g at Giza and 53.95 g at Sakha), while the lowest recorded for control treatment (40.62 g and 41.32 g for Giza and Sakha, respectively). For grain yield / Feddan , the yield was increased significantly in plants pretreated with pine oil, Hattric and clove oil being (17.95, 15.95 and 15.74 ardab, respectively) at Giza and (17.99, 16.31 and 15.98 ardab, respectively) at Sakha. However, tla least values for grain yield were recorded for control treatment being (11.19 ardab at Giza and 11.26 ardab at Sakha).

DISCUSSION

Loose smut, caused by *Ustilago nuda*, occurs wherever cultivated barley, it is more common in regions with a cool, moist climate during host flowering, the disease is not devastating, but causes low to moderate losses. However, even in dry, warm climates economic losses occur. The highly susceptible cultivars have losses in excess of 30% due to the high infection percentage **(Oort, 1944)**. Barley losses of 15 to 25% can occur in the absence of proper management practices **(Van Oosterom *et al.*, 1992).** In the same trend, **Tolessa *et al*. (2015)** reported that 20% loose smut severity on major

Table 7. Effect of nine effectors on some plant parameters of barley inoculated with loose smut fungus under field conditions at Giza and Sakha locations during 2020/21 to 2021/22 growing seasons

Effector	Grain yield /Fed. (Ardab)		1000-grain weight (g)		Grain weight / spike (g)		No. grains /spike		Plant height (cm)	
	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza
Hattric	16.31	15.95	51.32	51.33	2.69	2.63	58.65	56.67	105.43	99.23
Raxil	15.75	14.99	48.33	49.23	2.76	2.71	59.30	57.00	102.57	97.37
Simasol	13.97	13.64	49.40	50.00	2.47	2.45	56.79	54.65	98.67	96.47
Clove oil	15.98	15.74	47.37	48.57	2.89	2.82	60.49	58.47	105.20	103.10
Pine oil	17.99	17.95	53.95	53.50	2.98	2.58	62.73	60.53	109.80	105.70
Castor oil	14.52	13.74	47.92	47.12	2.53	2.20	61.39	59.33	100.29	98.27
G. ray 150 Gy)	13.94	13.64	45.97	48.57	2.30	2.42	52.27	52.33	99.20	97.40
G. ray(200 Gy)	13.83	13.24	44.63	45.83	2.27	2.33	51.30	50.97	93.39	95.37
G. ray(250 Gy)	13.36	13.15	43.60	42.80	2.19	2.10	50.33	50.73	91.47	93.27
Control	11.26	11.19	41.32	40.62	2.11	1.93	40.65	41.67	84.50	81.60
L. S. D (5%)	2.23	2.11	6.29	6.14	0.50	0.54	9.97	9.45	11.03	10.07

cereal crops (including barley) in Borana Zone, Ethiopia. However, barley yield losses of 10 to 30% due to barley loose smut are still common in some countries **(Zang, 2017; Bekele *et al.*, 1994)** showed an incidence of 28% for barley loose smut in Western Amhara, Ethiopia. Furthermore, a field survey conducted in Awi, South Gondar and West Gojjam Zones of Ethiopia in 2014 indicated that barley loose smut incidence ranging from 4.04 to 10.64% at field level **(Wallelign *et al.*, 2015).**

Ustilago nuda has been also found on all wheat varieties, some races that are specialized on bread or durum wheat **(Nielsen, 1985)**. Likewise in nature, seven new hosts for *U. nuda* were recorded by **(Nielsen, 1978)** on *Agropyron* spp and *Hordeum* spp. He added that on all these hosts, the sori of *U. nuda* were covered by a thin membrane or peridium whereas the sori of *U. tritici* were naked.

Systemic infection of loose smut (*Ustilago nuda*) on barley occurs by penetration of ovary shoulder, the fungus grows through the seed coat into the scutellum and embryo at the base of the seed. After seed germination, the mycelia permeate the crown node and enter the growing point of the tillers, the fungus is carried passively up with the plant growing point, which eventually develops into a smutted ear **(Malik and Batts, 1960).** Therefore, the most widely, used method of control for loose smut is seed treatment, it's very important that the systemic fungicide used to treat the seed and not just external. Infected seed or developing plants do not show any visible symptoms until infected ears appear.

Also **Batts (1955) and Shinohara (1976)** reported that teliospores enter the floret, germinate and form dikaryotic hyphae that infect ovary, usually at the brush end, hyphae grow intracellularly, but in the integument and nucellus, the fungus grows intercellularly, mainly on the dorsal side of the developing caryopsis. The mycelium enters the upper and parts of the scutellum 10 to 15 days after penetration and grows through the hypocotyl into the phumular bud, or growing point of the

embryo, where it will lie dormant in the mature seed. When the seed germinates, the mycelium is revitalized and carried in the crown node as the sub crown internode elongation. The fungus permeates crown tissues and enters the initials of the inflorescence **(Batts and Jeater, 1958 a&b).**

Environment plays a significant role in development and spread of this disease, teliospores germination occurs at 22 to 27 ^{O}C, whereas relative humidity ranges from 60-90% **(Druzhin and Krupnov, 2000)**.

Excessive heat or dry air will lower germination and germ tube growth, delay penetration of the ovary and preclude the fungus from reaching the growing point. The environment can also cause florets to stay open for a shorter time, which will reduce spore entry **(Atkins *et al*., 1963).** This shows the difference in the results obtained at the two sites of Giza and Sakha where the weather conditions vary between the two sites.

Dry inoculation method was found most effective than wet method**,** this result are in agreement with the findings of **Grevel (1930)**, **Cherewich and Cunningham (1956) and Mishra and Jain (1968).**

Although technology is being researched and used to help speed the process of resistant variety development, these traditional breeding methods are still very slow, and it's difficult to develop varieties with resistance which also possess other desirable traits such as those for yield and grain quality.

The most widely used method of control of loose smut disease is using treated seed. It is very important that the systemic fungicides used to treat the seed be and not just external. It is evident from the study that pesticide use when applied on seed consistently gave very good control of loose smut of barley during years of trial **(Dharam and Maheshwarl, 2001)**. Although the application of fungicides is almost always effective, their non-target environmental impact and the development of pathogen resistance have led to the search for alternative methods, especially in the past few years.

The loose smut control values were higher efficiency by Raxil than of Hattric, however it did not show a significant difference in barleyyur plant height, grain-filling period, thousand seed weight hectolitre weights and there improvement in the disease control by integrated approachusing by using of Simasol and pine oil. In this direction, the use of simasol gave good results in the resistance of the loose smut, despite its efficiency, some researchers have reservations about its use, as some studies indicate that it is a carcinogenic compound **(Asimakopoulos *et al*., 2012)**.

Also, some natural oils such as pine oil and clove oil can also be used as a seed treatment against loose smut as an alternative to fungicides. However, further study is required to determine the rate of application, the mechanism of control, the chemical responsible for such activity and their chemical and physical properties.

REFERENCES

Agrios, G.N. (2005). Plant Pathology, Fifth edition, London: Elsevier Academie Press, Project Director's Report 1991-92, All India Coordinated Wheat Improv. Proj. Directorate of Wheat Res. Karnal, 8.

Asimakopoulos, A., N. Thomaidis and M. Koupparis (2012). Recent trends in biomonitoring of bisphenol A, 4-t-octylphenol, and 4-nonylphenol. Toxicol. Letters, 210 (2): 141–154.

Atkins, I.N., O.G. Merkle, K.B. Porter, K.A. Lahr and D.F. Weibel (1963). The influence of environment on loose smut percentage, reinfection and grain yields of winter wheat at four locations in Texas. Plant Dis., 47: 192-196.

Batts, C.C. (1955). Observations on the infection of wheat by loose smut (*Ustilago tritici* (Pers.) Rostr.). Trans. Br. Mycol. Soc., 38: 465-475.

Batts, C.C.V. and A. Jeater (1958a). The development of loose smut (*Ustilago tritici*) in susceptible varieties of wheat, and some observations on field infection. Br. Mycol. Soc., 41: 115-125.

Batts, C.C. and A. Jeater (1958 b). The reaction of wheat varieties to loose smut as determined by embryo, seedling, and adult plant tests. Ann. App. Bio., 46: 23-29.

Baik, B.K. and S.E. Ullrich (2008). Barley for Food: Characteristics, Improvement, and Renewed Interest. J. Cereal Sci., 48:233-242.

Bekele, H., T. Hailu, A. Melkamu, A. Mesele and Y.S. Paul (1994). Management of loose smut and leaf stripe of barley using seed dressing fungicides. In: Crop Prot. Soc. Ethiopia, Addis Ababa., 14 - 15.

Brown, J. K. (1996). The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol., 133: 181-195.

Cherewiek, W.J. and R.H. Cunningham (1956). Further improvement in the partial vacuum method of inoculation with loose smut fungi. Phytopthol., 46: 355-358.

Dharam, S. and V.K. Maheshwart (2001). Biological seed treatment for the control of loose smut of wheat. Indian phytopathol., 54 (4): 457-460.

Druzhin, A.E. and V.A. Krupnov (2000). Influence of environmental conditions for infection of spring wheat by loose smut in Volga region. Doklady Aossiiskoi Academie Selskokhozyaistvennykh Nauk., 2: 16-18.

Grevel, F.K. (1930). Studies on the presence of biological races of loose smut of wheat (*Ustilago tritici*). Phytopathol. Z., 2:209-234.

Gupta, A.M., S.S. Beniwal, R.B. Karwsra, P.C. Arora and S. Singh (1991). Source of resistance to loose smut (*Ustilago nuda*) of wheat, (*Triticum aestivum* and *T. Durum*). Indian J. Agric. Sic., 16: 150-151.

Joshi, L.M., D.V. Singh and K.D. Srivastava (1988). Manual of Wheat Diseases, Malhotra Publishing House, New Delhi, 75.

Malik, M.M. and C.C. Batts (1960). The development of loose smut of barley (*Ustilago nuda*) in the barley plant, with observations on spore formation in nature and in culture. Transaction of the British Mycol. Soc., 43: 126–131.

Menzies, J.G., T.K. Turkington and R.E. Knox (2009). Testing for resistance to smut diseases of barley, oats and wheat in Western Canada. Can. J. Plant Pathol., 31: 265–279.

Mishra R.P. and A.C. Jain (1968). Study on loose smut of barley. JNKVV. Res. J., 2:17-18.

Nelson, R., K. Abarenkov, K. Arsson and U. Kõljalg (2011). Molecular identification of fungi. Rationale, Philosophical Concerns, and the UNITE Database, The Open Appl. Inform. J., 5 (Suppl 1-M9): 81-86.

Nielsen, J. (1978). Host range of the smut species *Ustilago nuda* and *Ustilago tritici* in the tribe *Triticeae*. Can. J. Bot., 56:901-915.

Nielsen, J. (1985). *Ustilago* spp. pathogenic on *Aegilops*. II. *Ustilago tritici*. Can. J. Bot., 63: 765 - 771.

Nielsen, J. (1987). Reaction of *Hordeum* species to the smut fungi *Ustilago nuda* and *U. tritici*. Can. J. Bot., 65: 2024-2027.

Oort, A.J. (1944). Onderzoekingen over stuifbrand II. Overgevoeligheid van tarwe voor stuifbrand (*Ustilago tritici*). With a summary: Hypersensitiveness of wheat to loose smut. Tijdschr. Plantenz., 50: 73- 106.

Pandy, D.K. and P.L. Gautam (1988). Morphology of spike in relation to resistance to loose smut (*Ustilago tritici*) in wheat (*Triticum aestivum*). Ind. J. Agric. Sci., 58: 713-714.

Poehlman, J.M. (1945). A simple method of inoculating barley with loose smut. Phytopath., 35: 457-460.

Ramdani, A.M., M. Jlibene and M. Boulif (2004). Survey of wheat diseases in the North West region of Morocco during 1997-98. Al Awamia, 111: 330-340.

Rewal, H.S. and J.S. Jhooty (1985). Differential response of wheat varieties to systemic fungiloxicanis applied to control *Ustilago tritici* (Pers). Indian J. Agric. Sci., 55 (8): 548-549.

Saari, E.F., O.F. Mamluk and P.A. Hurnett (1996). Wheat bunts and smuts diseases of wheat. Concepts and Methods of Disease Management, Mexico, D.F: CIMMYT.

Sallam, M.E., M.M.A. Abou-Taleb and F.K. El-Nashar (2001). Evaluation of some plant and

mineral oils the control of leaf rust disease of wheat .Egypt. J. Phytopathol., 29: 1-17.

Sammonr, R.H., M.M. El Shamy, A.M. Mustafa and E.S.Y. El-Refaay (2015). Inheritance of resistance to loose smut *Ustilago tritici* of wheat . Res. and Rev. Bio-Sci., 10 (4): 137-146.

Shinohara, M. (1976). Behaviour of *Ustilago nuda* (Jens.) Rostr. and *U. tritici* (Pers.) Rostr. in their host tissues. Rev. Plant Prot. Res., 9: 124–142.

Snedecor, G.W. (1957). Statistical Methods, 5th eds. Iowa State College Press. Ames, Iowa.

Tolessa, T., B. Natol, S. Yonas and S. Addis (2015). Assessments of major cereal disease in potential agro-ecology of Borana Zone, Southern Oromia. J. Biol., Agric. and Healthcare, 5 (11): 119-123.

Wallelign, Z., D. Mashilla and A. Dereje (2015). Importance of loose smut [*Ustilago nuda* (Jensen) Rostrup] of barley (*Hordeum vulgare* L.) in Western Amhara Region, Ethiopia. East Afr. J. Sci., 9(1): 31-40.

Walters, D.R. and D.C. Murray (1992). Induction of systemic resistance to rust in *Vicia faba* by phosphate and EDTA : Effects of calcium. Plant Pathol., 41(4): 444-448.

White, T., T. Bruns, S. Lee and J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications, 18: 315–322.

Wunderle, J., A. Leclerque, U. Schaffrath, A. Slusarenko and E. Koch (2012). Assessment of the loose smut fungi (*Ustilago nuda* and *U. tritici*) in tissues of barley and wheat by fluorescence microscopy and real-time PCR. Eur. J. Plant Pathol., 133: 865–875.

Van Oosterom, E.J. and E. Acevedo (1992). Adaptation of barley (*Hordeum vulgare* L.) to harsh Mediterranean environments. Euphytica, 62: 1–14.

Zadoks, J.C., T.T. Chang and C.F. Konzak (1974). A decimal code for the growth stages of cereals. Weed Res., 14: 415-421.

Zang, W. (2017). Investigation of Un8-Mediated Barley Loose Smut Resistance. Ph.D. Dissertation, Dept. Plant Sci., Coll. Graduate and Postdoctoral Studies, Univ. Saskatchewan. Saskatoon: Saskatchewan, Canada, 150.

تعريف ومقاومة السلالات المرضية (*Ustilago nuda*) المسببة لمرض التفحم السائب في الشعير

بدوى رجب حسن مهدلى[١] - طارق السيد عبدالباسط[١] - محمد منصور عبدالعاطى[٢]
هبة جمعة على جمعة[٢] - سالى المرسى على الوكيل[٢] - خيرى عبدالعزيز عامر[٢]

١- معهد بحوث أمراض النباتات - مركز البحوث الزراعية - مصر

٢- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية - مصر

يعتبر تعريف *Ustilago nuda* (Jens.) Rostr العامل المسبب لمرض تفحم الشعير السائب باستخدام الواسمات العشوائية متعددة الأشكال أسرع وأسهل من استخدام الطرق التقليدية (الأصناف المفرقة)، حيث تحديد الأجناس الفيزيولوجية المنطقية قد يحتاج إلى عدة سنوات. ولقد تم اختبار عدة مؤثرات في موقعي محطتي البحوث الزراعية بالجيزة وسخا لمعرفة مدى فاعليتها في تخليص حبوب الشعير المصابة من الفطر المتواجد بداخلها، عن طريق النقع في بعض الزيوت الطبيعية، والتعريض لأشعة جاما، والمعاملة ببعض مبيدات الفطريات الجديدة، ظهر من بين تسعة مؤثرات تم اختبارها، اثنان فقط هما (Simasol و Raxil) كان لهما أعلى درجة من الفعالية (تصل إلى ١٠٠%)، وكان يليهما Hattric وزيت الصنوبر بفعالية (٩٨.٤ و ٩٧.٩% على التوالي)، كما غيّر التعريض لإشعاع جاما عند ٢٥٠ جراي متبوعًا بزيت القرنفل وزيت الخروع ردود الفعل إلى مقاومة معتدلة ضد *U. nuda*. سببت إصابة بذور الشعير اختلافات معنوية بين جميع الصفات النباتية المدروسة.

المحكمـــون:

١- أ.د. يوسف السعيد عرب أستاذ أمراض النباتات – كلية الزراعة – جامعة الأزهر.

٢- أ.د. دولت أنور عبدالقادر أستاذ أمراض النبات المتفرغ – كلية الزراعة – جامعة الزقازيق.