J. Plant Prot. and Path., Mansoura Univ., Vol.5 (4): 379 - 392 , 2014

ENHANCING VIABILITY OF ONION SEED USING SOME TREATMENTS

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ABSTRACT

This study was performed at Seed Technology Research Unit, Mansoura. Seed Technology Research Department, ARC, to evaluate the efficiency of treating onion seed with Vitavax-thiram200,Bio-zeid,Bio-arec and Salcilic acid on seed germination, seedling vigor and seed-borne fungi of the tested cultivars(Giza Red, Giza 20,Giza 6 and Farmer seed) by blotter method.*Alternaria* sp., *Aspergilus* spp., *Botrytis allii*, *Drechslera* sp., *Epicoccum* sp., *Fusarium* spp., *Pencillium* spp., *Rhizoctonia solani* and *Rhizopus* sp.were associated with onion seeds.Treatments of onion seeds with the Fungicide (Vitavax-200), Biotic (Bio-arec and Bio-zeid) and Abiotic (Salicylic acid) showed that the fungicidal treatment Vitavax-200 with recommended doses eliminated the mentioned fungi completely, but gave the lowest mean percentage of germination (MPG). Biotic gave the highest of MPG with all cultivars as compared with the control. Abiotic played an important role in enhancing seed viability with all cultivars and increased seedling length, especially root length when applied as seed soaking. Cultivar Giza 20 gave the highest (MPG) and vigorous followed by cv. Giza Red, cv. Farmer seed and cv. Giza 6. Bio-arec and Bio-zeid gave protection and less numbers of total pathogenic fungi of onion seed with high significant, salicylic acid reduced of diseases incidence in seedlings.
Keywords: Onion seed-viability-fungi-fungicides-biotic-abiotic

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important field crops grown and used through the world. It has been used as an ingredient in various dishes for the thousands of years by many cultures around the world.Seed production is a vital part in onion growing. Seed borne fungi of onion seeds play an important role with this part especially during of storage and germination. Five species of *Aspergillus*, two species of *Fusarium* and each from *Pencillium*, *Drechslera*, *Curvularia*, *Rhizopus* and *Alternaria* were associated onion seed (Swatie *et al.,* 2011a).Ali, *et al*. (2002) found that leaf blotch and blast were to be the major problem of onion in the field, whereas black mold rot caused by (*Aspergillus niger*) was observed as the major pathogen in the storage.A number of fungi are associated with onion seeds, which can be controlled by seed treatment with Vitavax (Carboxin). *B. allii*,*B.cinerea, Fusarium* spp*. and Pleospora herbarum* were associated on onion seeds, seedlings and bulbs,where as *Alternaria porri* only on seeds. The level of seed infection by *B.allii* was lowest whereas bulb infection in store and after storage was highest (Tylkowska and Dorna, 2001). *Aspergillus niger* (black mould) reduced seed germination, emergence and distorted seedling growth (El-Nagerabi and Ahmed, 2001). Seed-borne microorganisms were the most effective on seed viability and vigour with high significance. *Fusarium* and *Pleospora* were the most frequently found

pathogens,fowllwed by *B.allii* (Tylkowska and Dorna, 1996). Ahmed *et al*., (1992), found that *Alternaria* spp. and *Rhizopus* spp.were only carried externally while *B. allii* was only borne internally, the other fungi associated with onion as seeds borne were *Aspergillus niger* and *Pencillium* spp. found internally and externally. Percentage infection with *B.allii* caused by neck rot was increased by increasing the inoculum potential. Abd-El-Razik, *et al*., (1990) in Egypt isolated *Fusarium oxysporum F.moniliform*e and *Rhizoctonia solani* were pathogenic and caused pre- and post-emergence damping-off of onion seeds. The fungicides Bavistin and Benlate are the most effective in reducing fungal growth and inhibited it on PDA medium and protected seedlings from infection in green house trials.*Aspergillus niger* causing (black mould) was transmitted from contaminated seeds giving seedlings with longer roots but shorter shoots than those grown from healthy seeds. Seed treatment with either Benomyl plus Thiram at the rate of 2.5 + 2.5 g / kg seed or in hot water (15 min at 60 degree C^{o}) eliminated the fungs in a naturally infected seed stock, untreated seed of which produced 30 % infected seedlings (Hayden and Maude, 1992). Seed-borne fungi,*A.niger, Dreshslera* sp*., Fusarium oxysporum , Pencillium* sp*., C.lunata* were isolated by blotter paper method then treated seeds with bioagents (*Trichoderma virdi* and *T.harzianum*) and fungicides, Carbandazim, Benlate, Thiram and combination treatment of (Carbandazim + Thiram) to control seed borne fungi, improving germination, shoot and root length, seedlings vigour index and pre - and post - emergence mortality of onion (Swati, *et al*., 2011 b).Singh *et al*., (1996) detected in comparison between fungicides, Thiram, Bavistin, Jkstein Diathane M-45, Emison and Topsin-M to control storage diseases.*Aspergillus niger*,*A.alternate, Rhizopus* and *Fusarium* were absent on treated seeds .Thiram was the most effective fungicide fowllowed by Bavistin. Beratonli, *et al*.,(1996)found that *Bacillus megaterium* inhibited *Rhizoctonia solani* transmitted by seeds as biocontrol. Abeer El-Saeed, (2004) pointed that the highly significant decrease in the percentage of infection by *A.niger* and *Fusarium oxysporum* on onion bulbs occurred by using *T.harzianum* followed by *T.virdi* and *T.hamatum*. Ibrahim, (2009) found that the biocide (Bio-arec and Bio-zeid) lead to highest percentage of normal seedlings from 76 to 96 % and lowest dead seeds from 41 to 4 % and reduced abnormal seedlings as treatments with wheat seeds. The aim of the present study was enhancing seed viability of onion seeds by using fungicide, biotic and abiotic treatments.

MATERIALS AND METHODS

In the present study on samples from Onion Seed Research Department, Field Crops Research Institute. Agricultural Research Center, except Farmer seed which obtained from Salaka village, Dakahlia governorate. The seed was investigated by blotter method, recording percentage of seed-borne fungi after treatments with Fungicide, Biotic and Abiotic. Also, the effect of these treatments on germination, vigor and viability in Petri-plate was studied.

Naturally infected seeds of onion seed four cultivars cv. Giza 6, cv. Giza 20, cv. Giza Red and cv. Farmer seed used for these work in season 2013 for two times at the Laboratory of Seed Technology Research Unit , Mansoura, Egypt .

1-Fungicide treatments:

Onion seeds were used in this experiment for each cultivar were treated with Vitavax-Thiram -200 (Carboxin 37.5 % + Thiram 37.5 %) as a wet powder for seed dressing treatment at the rate of 2.5 g / kg in 100 ml dry flasks on a mechanical shaker for about 20 minutes. Two hundred seeds were used following the standard blotter method in eight replicates (25 seeds / petri dish, 9 cm). Other two hundred seeds treated with water only from each cultivar were plated as a control treatment were incubated at 20 $C^{o}\pm$ 2 for seven days under alternating cycles of 12 hours near ultraviolet (NUV) light and darkness. Percentages of fungi under investigation were calculated by stero-binocular microscope and recorded by the blotter method according to the following formula:

Fungal % $= \frac{N1-N2}{N1} X100$

N1 =the number of treated seeds.

N2 = the number ofseeds with fungal growth.

Viability test were determined by placing 100 seed treated with the fungicide (Vitavax-Thiram) as previously mentioned from each cultivar. Seeds were planted on No 3 Whattman filter paper (25 seeds per 12 cm petri plate) wetted with 10 ml of sterile water and incubated for 12 days at 20 C^{O}. Also, to ensure continuous high humidity, the germination plates were packed in a clear plastic bag (4 plates per bag). On the other hand, one hundred untreated seeds served as a control and observed daily to study the following characters:

Percentages of germination, shoot length, root length and dry weight of seedlings (ISTA rules, 1999).

MPG $= \frac{N1-N2}{N1} X100$

The mean percentage germination (MPG), N1 is the number of treated seeds plated, N2 the number of un germinated seeds.

- **Seedling vigor index** = MPG ×Seedling length.
- **Vigor test** = MPG × Seedling dry weight.

2-Biocides treatments of onion seeds:

Two different biological preparations namely Bio-zeid and Bio-arec obtained from Biological Control Unit, Plant Pathology Research Institute the preparation contains active ingredient biocides were used at rate of 5gm / kg seed of onion seed cultivars under study.

Onion seeds of each tested cultivar were treated with the mentioned two biocides using 5gm biocide per each 1kg of seeds plus 3 ml of sterile water, all were mixed properly and air dried for 30 minutes on sterile tray to enable the seeds to absorb the biocides. Two hundred seeds used for each

treatment in eight replicates each of (25 seeds / petri-dish, 9 cm). The control was soaked in sterile distilled water only and air dried with the same way, as the other treatments were incubated at 20 C^{o} ± 2 for seven days, then were examined under stereo-binocular microscope. The fungal infection observed was identified and characterized as previously mentioned. The effect of treatments with biocides on viability of the seeds was determined as fungicide treatment.

Table (1): Bio-agent used in seed dressing of onion seeds.

Commercial name	Bio-agent	Concentration
Bio-zeid	*Trichoderma hamatum (album)*	10×10^{6} spore/gm.
Bio-arec	*Bacillus megaterium*	25×10^{6} CFU /gm.

3-Abiotic treatment:

5gm, of onion seeds for each cultivar were soaked in salicylic acid concentration of 50 ppm for two hours. Two hundred seeds were plated on the three layers blotter paper, 25 seeds were plated at equal distances. On the other hand two hundred seeds treated with distilled water as check. All these petri-plates were incubated at 20 C^{o}± 2 under 12 hours cycle of alternate light and darkness **(ISTA, 1999).** After 7 days of incubation, petri-plates were observed under sterio-binoculear microscope for having the percentage fungal infection as previously mentioned .The effect of the treatment by salicylic acid (SA) on the viability (shoot length, root length and dry weight) were done as previously mentioned .

4- Statistical analysis:

The observed data were statistically analyzed as the technique of analysis of variance (ANOVA) of the randomized complete block design as mentioned by **(Gomez and Gomez, 1984).** The means were compared using the least significant differences (L.S.D). Statistical analysis was performed using analysis of variance technique (ANOVA) by means of "MSTAT-C"computer software package.

RESULTS

1-Effect of treatments under study on onion seed viability:

High significant results are shown in (Table 2) characters viability of the tested cultivars, cv. Giza 20 recording the highest value of germination percentage (73.6 %) followed by cv. Giza Red (72.4 %), while cv. Farmer seed and cv. Giza 6 were less, On the other hand the Farmer seed cultivar gave more seedlings length, cv. Giza 20 and cv. Giza Red gave more vigorous seedlings and dry weight when compared with other cultivars. Significant increase in germination percentage was observed with all treatments especially salicylic acid (SA) (77.6 %) followed by Bio-zeid (71.8 %), Bio-arec (67.3 %) and Vitavax-200 (62.5 %) compared with the control (57%). Also, salicylic acid gave the highest value with seedling length as compared with other treatments.

Table (2): Effect of onion seed cultivars and treatments on germination percentage, root length, shoot length, seedling dry weight, seedling vigor index and vigor index.

Treatments	G %	root length (cm)	shoot length (cm)	seedling dry weight (gm)	seedling vigor index	vigor index
A- Cultivars						
Giza Red	72	2.7	6.3	0.02	665	1.2
Giza 6	57	2.2	6.2	0.02	497	1.0
Giza 20	74	2.3	6	0.02	603	1.2
Farmer seed	66	2.7	7	0.02	647	1.1
F. test	**	**	**	**	**	**
L.S.D at 5%	2.6	0.2	0.3	0.05	32	0.04
B- seed treatment						
Control	58	1.5	4	0.01	311	0.6
Vitavax-200	63	2.7	7	0.02	593	1.1
Bio - zeid	72	2.4	7	0.02	652	1.2
Bio - arec	67	2.4	6.3	0.02	583	1.1
Salicylic acid	78	3.4	8.2	0.02	877	1.5
F. test	**	**	**	**	**	**
L.S.D at 5 %	3	0.3	0.3	0.1	35	0.1
C- Interactions						
A × B	**	**	**	**	**	**

Table (3) shows the effect of treatments on mean percentage germination (MPG) of seed cultivars under study. Salicylic acid recorded the highest value of MPG giving (85.3 %) with cv. Giza 20 followed by Bio-arec. On the other hand Vitavax-200 recorded the lowest value of MPG with different cultivars, where recorded (49 %) with cv. Giza 6.

Table (3):Means of germination percentage as affected by the interaction between onion cultivars and seed treatments.

Cultivars	Control	Bio-zeid	Bio-arec	Salicylic acid	Vitavax-200
Giza red	65	74	68	81	75
Giza 6	49	66	58	64	49
Giza 20	63	77	73	85	70
Farmer seed	52	70	71	80	56
F. Test	**				
L.S.D at 5 %	5.7				

2- Effect of Biocides, Abiotic and Fungicide on seed- borne fungi on cultivars under study by blotter test:

Table (4) cv. Giza Red indicated that Biocides (Bio-arec) was able to eliminate *Fusarium* sp. and *Rhizopus* sp. with 100 %efficiency,while reduced incidence of *Alternaria* sp. from 16 to 10 % with effectiveness 38 % *Aspergillus* spp.from 56 to 22 %, *Botrytis allii* from 24 to 10 % , Bio-zeid lead to eliminate *Drechslera* sp. and reduced numbers of the fungi, *Alternaria* sp., *Aspergillus* spp*., B. allii, Fusarium* spp. and *Pencillium* spp. Abiotic (salicylic acid) eliminated fungal infection with *Alternaria* sp.*,B.allii, Epicoccum* sp.and *Fusarium* spp. with 100 % effectiveness and reduced *Aspergillus* spp. from 52 to 8 % and *Pencillium* spp. from 46 to 10 %. On the other hand Vitavax-200 (2.5 gm,/kg.seeds) was highly effective in all cases, eliminating with 100 % efficiency control on seed-borne fungi of all cultivars used in this experiment.

Data in table (5) cv. Giza 6 showed that treatment by (Bi-arec) able to eliminate the seed-borne fungi, *Alternaria* sp., *B. allii*,*Drechslera* sp., *Fusarium* spp. and *R. solani*, while reduced *Aspergillus* spp. from 52 to 18 % and *Pencillium* spp. from 46 to 12 %, Bio-zeid reduced *Aspergillus* spp. from 52 to 16 and *Pencillium* spp. from 46 to 22 % salicylic acid lead to eliminated the fungi *Aspergillus s*sp*., B. allii*, *Drechslers* spp., *Epicoccum* sp., *Fusarium*spp*., Rhizopus* sp.and reduced numbers of *A. niger and Pencillium* sp.as compared with the check.The percentages of effectiveness 100 % to Bio-arec were recorded by both fungi; *Alternaria* sp*., Aspergillus* spp*., Drechslera* sp*., Pencillium* spp*.,* and *Rhizopus* sp. , while reduced levels of infection with *B. allii , Fusarium* spp.and *R. solani* .

Table (6) Bio-zeid gave highly significant protection of seeds against, *Aspergillus* sp*., B. allii, Drechslera* sp*,* and *Pencillium* sp. with effective 100 %. Salicylic acid gave the highest effective with most fungi as compared with control and other treatments except Vitavax-200.

Table (7) cv. Farmer seed, salicylic acid was the best treatment followed by Bio-arec and Bio-zeid as protection against seed-borne fungi of onion with high significant, where salycilic acid lead to reduction of infection incidence by *B. allii, F.* spp.and *R.solani.*

Table (8) indicate that, cv. Giza Red gave the highest total infection of seed-borne fungi (198) but gave the highest (MPG) 65% as control, while cv. Giza 6 recorded the lowest (MPG) Salicylic acid gave the highest value MPG (86 %) cv. Giza 20 with total fungi (9) the less followed by Bio-zeid (77 %) and (18) total fungi, Bio-arec (73.0 %) (12). Vitavax-200 has the lowest MPG while lead to eliminate of fungi.

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Table (8):Effect the number total of fungi on mean germination percentage.

Cultivars	Control		Bio-zeid		Bio-arec		Salicylic acid		Vitavax-200	
	G%	T.fungi	G%	T.fungi	G%	T.fungi	G%	T.fungi	G%	T.fungi
Giza Red	65	198	74	100	68	56	81	26	65	0
Giza 6	48	153	66	50	58	30	65	18	49	0
Giza 20	62	100	77	18	73	12	86	9	63	0
Farmer seed	52	94	70	18	71	22	80	32	52	0

Table (9) show that Vitavax-200 was the most effectiveness on total fungi, while salicylic acid less total fungi from (141) as control to (21), Bio-arec to (30.5) and Bio-zeid to (47). Also, treatment with salicylic acid lead to reduced numbers of fungi *Aspergillus niger*, *B. alli, Fusarium* spp. and *Pencillium* spp. followed by Bio-arec and Bio-zeid.

Table (9): Effect of seed treatments on mean percentage fungi (MPF).

Treatments	*Alternaria* sp.	*Aspergillus* spp.	*Botrytis allii*	*Drechslera* sp.	*Epicoccum* sp.	*Fusarium* spp.	*Pencillium* spp.	*Rhizoctonia solani*	*Rhizopus* sp.
Control	12	39	15	4	11	12	34	5	9
Bio-zeid	5	16	6	0	0	3	15	1	1
Bio-arec	3.5	13	4	1	0	1	7	1	0
Salicylic acid	0	8	2	0	0	1	10	0	0
Vitavax-200	0	0	0	0	0	0	0	0	0

DISCUSSIONS

The standard blotter method (Seed Health Testing) revealed nine fungal isolates from onion seeds representing four cultivars. Among, the isolated fungi seed-borne pathogens were included in the present investigation namely, *Alternaria* sp., *Aspergillus* spp., *Botrytis allii, Drechslera* sp., *Epicoccum* sp., *Fusarium* spp., *Pencillium* spp., *Rhizoctonia solani and Rhizopus* spp.

In the present study seed-borne fungal infection could reduce seed viability by species, *Alternaria, Botrytis* and *Fusarium,*this results were in harmony with(Janas and Robak, 1999).Cultivar Giza 20 was the highest of MPG followed by cv Giza Red, cv.Giza 6and cv.Farmer seed respectively but Ahmed *et al*., (1992) reported that cv. Giza 6 was relatively more resistant than cv. Giza 20 to infection with *Botrytis allii* and could pass from seed to the resultant seedling, initially appearing in the cotyledon and subsequently in the true leaves or leaf bases.

The relationship between level of infection by *Botrytis allii* and bulb rot in store was established with higher seed infections resulting in greater levels of bulb rot (Stewart and Franicevic, 1994). Also, *Aspergillus* spp. was associated with onion seeds with the highest percentage of different cultivars,

Aspergillus niger (Black mould) reduced seed germination due to presumably to the toxic metabolites secreted by the fungus. They found that the red cultivars reduced seed infection pre - and post - emergence damping-off and enhanced the growth of the seedlings in the field (Elnagrabi and Ahmed, 2001). Seed-borne fungi*; Aspergillus niger, D*rechslera spp.*, F. oxysporum, P*encillium spp.*, Curvularia lanata* were isolated by blotter papper method. These results were in harmony with those reported by Swati, *et al*., (2011b)also found improve in germination , shoot and root length, seedling vigour index and pre- and post-emergence mortality of onion when treated seeds by bioagents (*Trichoderma virdi* and *T. harzianum*) and fungicides (Thiram) and combination of (Carbandazin + Thiram). The obtained results showed that fungicidal treatment Vitavax-Thiram with the recommended doses eliminated the fungi under test completely, whereas gave the lowset MPG as compared with other treatments because Vitavax-200 induces various types of spindle abnormalities, inhibits cell plate formation and exhibits antimiotic activity at a concentration of 500 mg/1 and above (Somasheker and Gowda, 1984). Biotic (Bio-zeid and Bio-arec) gave the highest of MPG with all cultivars as compared with control .These results were in harmony with those reported by Morsy *et al*., (2011)they found Bio-arec and Bio-zeid lead do provide protection against alfalfa downy mildow, rust, root rot and wilt diseases when applied as spry treatment or seed soaking. Biotic products able to reduce numbers of seed born fungi under study. These results are similar to the findings obtained by (Morsy *et al*., 2011) and (Ibrahim, 2009).Biological products can serve as an alternative to some chemical fungicides, especially in case of fungicide failure. The bacteria as shown by different species of *Bacillus* appear to protect plants against a wide range of pathogens and the potential for commercial utilization is promising. Fungal Bio-control agents, including the extensively studied *Trichoderma* spp. have been reported to reduce infection or reproduction of many pathogens. Abiotic (Salicylic acid) play an essential role in enhancing seed germination with all cultivars and increased in seedling length especially root length when applied as seed soaking as compared with other treatments, also reduction of diseases incidence in seedlings by seed-borne fungi. Salicylic acid is common plant produced phenolic compound, endogenous growth regulator of physiological process in plants. Exogenous application of salicylic acid may influence stomata closure ion uptake and transport inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan and Smith, 2003).Salicylic acid could induce systemic resistance in chickpea against *Fuzarium* wilt disease and seed dressing is the best and practicable method of application in the field (Sarwar, *et al*., 2010).

Generally, data obtained through this work show that treatments of onion seed with biotic and abiotic lead to improving seed viability and reduction of fungal diseases as a safe way recommended in controlling.

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تحسين حيوية تقاوى البصل باستخدام بعض المعاملات

السيد احمد محمد إبراهيم و عبدالمجيد محمد سعد كشك

معهد بحوث المحاصيل الحقلية- قسم بحوث تكنولوجيا البذور- مركز البحوث الزراعية – مصر

اجريت هذه الدراسة خلال عام 2013 بتصميم التام العشوائية فى أربع مكررات للوقوف على إحدى المشكلات المؤدية إلى خفض فى حيوية تقاوى البصل وهى الفطريات الممرضة المحمولة على تلك التقاوى بعزلها والتعرف عليها ومكافحتها ظاهريا وحث البادرة على المقاومة وذلك مع أصناف جيزة أحمر -جيزة 6- جيزة 20 وتقاوى المزارع وتم إجراء هذه الدراسة مرتين فى معمل بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية حيث تمت معاملة تقاوى الأصناف السالفة الذكر بالمبيد الفطرى فيتافاكس- ثيرام بمعدل 2.5جرام / كجم بذرة ومنتجات حيوية هى: Bio-arec, Bio-zeid بمعدل 5 جم /كجم بذرة ونقع تقاوى البصل المستخدمة فى محلول حمض السالسليك بمعدل 50 جزء فى المليون لمدة ساعتين وكانت النتائج كالأتى:

- تم عزل وتعريف فطريات جنسألترناريا- أسبرجلس- بوترايتس- دريشسليرا- ابيكوكم- فيوزاريم - بنسليوم- رايزوكتونيا و ريزوبس كانت ملازمة لمعظم تقاوى الأصناف المذكورة .
- كانت أنواع فطريات بنسليوم - أسبرجلس-بوترايتس-فيوزاريم أكثر إنتشاراً حيث ادت تلك الفطريات إلى خفض فى نسبة الإنبات وحيوية البادرات.
- المعاملة بمبيد الفيتافاكس أدت إلى إقصاء تام للفطريات ولكن كان هناك إنخفاض ملحوظ فى نسبة الإنبات وكانت المعاملة بحمض السالسليك أحسن المعاملات خفضاً لعدد الفطريات على البذور يليه Bio-arec ثمBio-zeid.
- أدت معاملة التقاوى للأصناف المذكورة بالنقع فى محلول حمض السالسليك إلى تحسن فى نسبة الإنبات وزيادة فى طول البادرات يليه Bio-zeid ، Bio-arec وكان أقلهم مبيد فيتافاكس ثيرام حيث أثر سلبيا على تلك النسبة.
- أعطى الصنف جيزة 20 أعلى متوسط لنسبة الإنبات يلية جيزة أحمر ثم تقاوى المزارع وجيزة 6 وذلك فى جدول التفاعل بمعنوية عالية.

لذا توصى هذه الدراسة بإستخدام أصناف جيزة 20 ثم جيزة أحمروجيزة 6 محسن ثم تقاوى المزارع على الترتيب حسب مدى إعطائهم نسبة الإنبات والحيوية وإستخدام حمض السالسليك حيث يؤدى إلى حث البذور على الإنبات ومقاومة الفطريات المحمولة على التقاوى يلية استخدام المنتجات الحيوية لما لها من القدرة على خفض أعداد الفطريات المحمولة على التقاوى وكموادآمنة على البذور والبيئة.

قام بتحكيم البحث

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J. Plant Prot. and Path., Mansoura Univ., Vol.5 (4): 379 - 392 , 2014