

EFFECT OF PLANT EXTRACTS ON SEED QUALITY OF SOME FORAGE CROPS DURING STORAGE

Ibrahim, Abeer El- Ward A.; I. F. Mersal and M. I. El-Abady
Seed Tech. Res. Sec. Field Crops Res. Institute, Agric. Res. Center.

ABSTRACT

Maintenance of seed quality during storage until planting is imperative to assure its planting value and increasing seed yield. Laboratory experiment was carried out at Mansoura Seed Technology Research Unit, Seed Technology Research Section, Field Crops Research Institute, Agricultural Research Center, Egypt during 2008 and 2009 years to evaluate bioassay effects of (Ramith and Oshar) chlorophorm and ethanol extracts on *Bruchidius trifolii* (Poc), and the effect of treating seed of Egyptian Clover (*Trifolium alexandrinum* L.) c.v. helaly, Alfalfa (*Medicago sativa* L.) c.v. Ismaelia 1 and Fahl berseem with these plant extracts on seed quality and insect infestation during different storage periods (0, 6, 12 and 18 months). The results revealed that Ramith chlorophorm and Oshar ethanol extracts were the most toxic for (*Bruchidius trifolii*) followed by Oshar chlorophorm and Ramith ethanol extracts and the values of Lc50 were (4753.9, 8514.3, 11026.9 and 20649.6 ppm), respectively. After six months from storage, the germination percentage for both of Alfalfa, Egyptian clover and Fahl berseem seeds pass the minimum level for the acceptance of certified seed (85%). Insect inspection cleared, the insect infestation level in Alfalfa seed (4.45%) was the less, followed by both of Egyptian clover and Fahl berseem (6.4 and 6.2%). Treated seed with Ramith chlorophorm extract, produced high germination percentage, seedlings vigour and the lowest level of insect infestation (3.75%) comparing with untreated seed while its germination percentage reduced less than the acceptance level for certified seed and the insect infestation level increased to (8.67%). After storage with 18 months, germination percentage reduced gradually and becomes less than the acceptance level for certified seed and Alfalfa was the less in reduction of seed germination and seedling vigour traits. In general, comparing with the previous storage periods, seed quality decreased and insect infestation increased and reached to 22.67% for untreated seed. Storage periods negatively and significantly correlated with both of germination %, accelerated aging germination % and seedlings vigour index and highly positively correlated with insect infestation % and electrical conductivity. Also, insect infestation % negatively and highly correlated with germination % and seedlings vigour index.

This study suggested, using seed protectants such as Ramith chlorophorm and Oshar ethanol extracts for protect Alfalfa, Egyptian clover and Fahl berseem seed against *Bruchidius trifolii* (Poc). Also, planting (Alfalfa, Egyptian clover and Fahl berseem) certified seed in the same production year is preferable well but Clover carry over seed should be stored at optimum conditions and when planting seed rate should be increased to redress the reduction in seed quality.

INTRODUCTION

Because of its importance among forage crops in Egypt, Egyptian clover (*Trifolium alexandrinum* L.) has the rank of Alfalfa (*Medicago sativa* L.) as queen of forage crops in the world (Li *et al.*, 2007). However, Fahl berseem is characterized by a short vegetative cycle and no growth after cutting (Poenheimer, 1959). As legumes fodder crops in tropical and subtropical countries, they are considered favorable food source for a large

number of insects species. Many of these species are considered pests because of injury resulting from their feeding on different parts of the plant particularly seed.

The management of pest using synthetic chemicals has failed due to insecticidal resistance (Mehrotra ,1993) and caused extensive damage of both health and environmental. These problems clearly indicated that basic research must be directed to discover a new safe types of pest control agents. Many investigators in different parts of the world initiated large screening efforts to find plant extracts which have interesting physiological and miticidal affect (Abo El- Ghar *et al.*,1990 ; Pacheco *et al.*,1995 ; Tembo and Murfih, 1995 ; El-Lakwah *et al.*, 1999 ; Abd El-Wahab ,2003 and Mersal *et al.*,2009) without adverse effects on seed germination or plant growth (George and Patel, 1992). Zedan *et al.*(2007) reported that natural products affecting pests ,therefore provide continual inspiration to the agricultural chemicals in their research for new products to control pests and improve field.

Rimth (*Haloxylon salicornicum* L.) is worldwide plant distributed especially in desert and semidesert areas in soil containing much salt (Mabberley ,1997 and Evans, 2001) .It is reported to be used for diabetes (Ajabnoor *et al.* ,1984) ,as antiseptic and anti-inflammatory (Al-Shanawani ,1996) and larvicidal activity (Sathiyamoorthy *et al.*,1997). Salama and Ismail (2005) reported that Rimth has superior toxicity as compared to the extracts of the other tested plants estimated while LC50 were 0.978% . Oshar plants (*Calotropis procera*) has an active compound protect it from pests, insects and microorganisms (Akinloye *et al.*, 2002) had greater insecticidal activity, gave significant mortality (Chaudhry,1992; Khan and Siddiqui,1994 and Meshram,2000). Abbassi *et al.* (2003) reported that alkaloids extracted from Oshar prevent sexual maturity and there were a significant mortality for the desert locust (*Schistocerca gregaria*).

During seed storage, temperature and seed moisture content are the most important factors controlling seed longevity (Vijay *et al.*,2009) however, pests specially insects such as beetles of the family of *Bruchidius alferii* adapted to attack the mature seed causing extensive loss. Srivastava and Pant (1989) and Ramzan *et al.*(1990) reported that Bruchids (*Callosobruchus spp.*) are important storage pests of grain legumes, and are known to cause considerable economic losses, especially in pulses grown in the tropics and sub-tropics. Bruchid-damaged seeds do not germinate because of feeding on both of the endosperm and embryo. Keals *et al.* (1997) reported that *Bruchidius trifolii* has been shown to reduce the seed carry over in trail plots by 50%. While, Mersal *et al.* (2006) mentioned that the decrease in seed viability from insects because its feeding on the endosperm and embryo of seed .

The aim of this study was to investigate the effect of the two tested plant extracts in different solvents on *Bruchidius alferii* and their impact on stored seed quality.

MATERIALS AND METHODS

This investigation was carried out at the laboratory of Mansoura Seed Technology Research Unit, Dakahlia Governorate during 2008 and 2009 years to study the role of some plant extracts in protecting seed of some forage crops namely Egyptian clover (*Trifolium alexandrinum* L.) c.v. Helaly, Alfalfa (*Medicago sativa* L.) c.v. Ismailia 1 and Fahl berseem from storage insects (*Bruchidus trifolii* (Poc) as well as its effects on seed quality and insect infestation during different storage periods (0, 6, 12 and 18 months).

Plant materials and bioassay efficiency:

Table 1, clear the English , Latin , family names in addition to parts used and source of collection for the studied plants as insecticides.

Table 1 : Tested plant materials

English name	Latin name	Family name	Tested part	source
Oshar	<i>Calotris procera</i>	Asclepiadaceae	Aerial parts	El-Arish
Ramith	<i>Haloxylon salicornicum</i>	Chenopodiaceae	Aerial parts	El - Arish

Plant samples were collected during spring 2007 and identification were bases mainly on the taxonomic characters detailed by Tackholm (1956).

The tested plants were extracted according to the procedures outlined by Freedman *et al.* (1979) with some modification. The aerial parts of the tested plants were dried and grinded using laboratory grinder into fine powder and 200 g of powder were extracted three times successively with two solvents varied in their polarity . Chloroform was the first solvent used followed by ethanol . The homogenous extract was allowed to stand for three days and extracts were filtered through an hydrous sodium sulphate , combined and the solvent was evaporated under vacuum at temperature degree not accessed 50 ° C . The crude extract was then weighed and adjusted to 25 ml with the used solvent and kept in Refrigerator until testing.

The tested insects of *Bruchidus trifolii* (Poc) were identified in the Plant Protection Research Institute Agricultural Research Center, Dokki , Giza , Egypt. The treated surface exposure method of bioassay was used to evaluate the toxicity effect of the plant extract against *bruchidius affierii* (pic) .Serial concentrations of crude plant extract were prepared then 1 ml of each concentration was spreaded on the bottom of dishes . After the solvent had evaporated , newly hatched insects were placed in each dish covered and kept at room temperature for 24 hours , then mortality percent was counted. three replicates with 10 individuals were used for each concentration in addition to untreated check (control) free from toxicant. Mortality readings was corrected according to Abbott (1925) and all the obtained data were subjected to statistical analysis to evaluate the relative efficiency of the tested plan insecticides according to Finney (1952).

Seed samples of Egyptian clover, Alfalfa and Fahl berseem were obtained from Forage Crops Research Sec. FCRI, ARC. And they were sieved and cleaned from any inert materials and treated with the following plant extracts concentration in addition to the check treatment (untreated).

Ramith chlorophorm extract(Lc50= 4753.9), Ramith ethanol extract (Lc50= 20649.6), Oshar chlorophorm extract (Lc50= 11026.9) and Oshar

ethanol extract (Lc50= 8514.3). Then, the treated samples were dried in open air and the studied traits were recorded, other samples treated and stored in cloth bags in open air under the laboratory conditions for 6 months and others treated and stored for 12 months and others for 18 months.

Studied traits:

1- Seed quality traits:

Germination percentage: Germination percentage was performed according to ISTA , 1985, while 400 seeds of clover in 4 replicates were sown at 20 ° C \pm 2 in sterilized sand culture . Germination percentage defined as the total number of normal seedling at the end of the test after eight days.

Seedling length (cm): During the final count 10 normal seedlings from each replicate were taken randomly to measure the seedling length.

Seedling dry weight: Ten normal seedlings were dried in a hot-air oven at 85°C for 12 hours (Kirshnasamy and Seshu, 1990) and then weighted in (g).

Seedling Vigour Index: Seedling vigour index was calculated with the help of data recorded on germination percentage and seedling growth according to International Seed Testing Association (ISTA, 1985) by the formula :

$$S. V. I. = \text{Seedling dry weight (g)} \times \text{Germination percentage.}$$

Accelerated aging germination test(germination after seed aging).

Accelerated aging test was performed according to (ISTA, 1985). Each sample of 400 seeds were placed in an accelerate ageing chamber at 40° C and 100 % relative humidity for 48 h. After this exposure period, the seed sample was submitted to the Standard Germination Test.

Electrical conductivity (μ mohs / g seed). It was calculated according to Matthews and Alison (1987). Fifty seeds in four replications were weight to 2 decimal places and placed in a 200 ml flask and 67.5 ml of distilled water was added . The flask were covered and placed in an incubator at a constant temperature of 20 ° C for 24 hours , after which the contents of the flasks were gently stirred. The electrical conductivity was measured in the solution after removing the seeds. The HANNA conductivity meter (Hi 80333) was used. The results were reported as μ moh per one (g) of seed (μ mohs / g seed).

2- Insect infestation percentage.

Each sample after each storage period (6, 12 and 18 months) was inspected while, four replication of 100 seeds from each sample were used to estimate insect infestation. The infestation level was expressed as percent damage seed according to Jood *et al.* (1996).

$$\text{Insect infestation \%} = \text{No. infested seed} / \text{No. of inspected seed} \times 100.$$

Date were statistically analyzed as the technique of the ANOVA for the Complete Block Design. The treatment and means were compared using the least significant differences (LSD) according to Gomez and Gomez(1984).

RESULTS AND DISCUSSION

The action of the tested plant extracts revealed a great variation in its effectiveness against *Bruchidius trifolii* (Pic), as indicated in Table 2. Ramith cholorophorm extract exhibited the highest toxic action against *Bruchidius trifolii* (Pic), followed by Oshar ethanol extract, Oshar cholorophorm extract and Ramith ethanol extract, values of Lc50 were 4753.9, 8514.3, 11026.9

and 20649.6 ppm, respectively. Obviously, Ramith chlorophorm extract was more toxic than the ethanolic Ramith extract. These results are in agreement with those obtained by Akinloye *et al.* (2002) ;Chaudhry (1992) and Khan and Siddiqui (1994) while they reported the effectiveness of Ramith and oshar extracts in protection from pests (insects and microorganisms). Also El-Rokh (2007) reported that Ramith chlorophorm extract was more active than Ramith ethanol extract. On the other hand, ethanolic oshar extract was more toxic than its chlorophormic extract. Abbassi *et al.* (2003) reported that Alkaloids extracted from Oshar prevent sexual maturity and there were a significant mortality for the desert locust (*Schistocerca gregaria*).

Table 2: Lc 50, Lc90 and confidence limits at 95% (ppm) of four crude extracts against of *Bruchidius trifolii* (Pic), (24 h. post treatment).

Plant extracts	Material	LC ₅₀ and confidence limits at 95%			LC ₉₀ and confidence limits at 95%			Slop value
		LC ₅₀	Lower	Upper	LC ₉₀	Lower	Upper	
Ramith chlorophorm		4753.9	4119.5	5486.0	12773.6	11069.0	14740.8	2.99
Ramith ethanol		20649.6	16454.3	30440.0	94503.7	53665.4	292390.0	1.83
Oshar chlorophorm		11026.9	9131.0	13599.4	55350.6	35134.1	133530.0	1.84
Oshar ethanol		8514.3	6832.5	10251.1	42444.6	28418.6	91536.1	1.94

Data presented in (Table 3) show the variations of seed quality (seed germination and seedling vigour) among the Egyptian clover, Alfalfa and Fahl berseem, as effected plant extracts directly after treatment (before storage) . Significant differences between the three tested forage crops in seed and seedlings vigour traits. Alfalfa seed have the highest germination percentage (98%), accelerated aging germination (74%), seedlings vigour as measured by seedling length (10.9 cm), seedling dry weight (0.053g)and seedling vigour index (5.1). Meanwhile, it recorded the lowest reading (0.155µs/cm/g) of electrical conductivity for leached seed. On the other hand, germination percentage before and after aging for Fahl berseem were the lowest (95% and 67%) as comparing with (96% and 71%) for Egyptian clover. Although, germination percentage for the clovers passed the minimum standard acceptance level for certified seed (85%) but, these variations in seed germination and seedlings vigour before storage may affect seed germination with prolonging the storage time. While seeds that gave high initial viability maintain their quality for longer period than seeds with low viability and therefore it is necessary to know what seed lot has high viability (good candidate for storage) and which have low viability (higher risk in storage) Elias *et al.* (2009).

The studied traits (directly after treatment) significantly affected with plant derived material extracts except seedlings length as presented in (Table 3). Treated seed with both Ramith ethanol and Oshar chlorophorm extracts produced the highest germination percentage (97%). Meanwhile, treated seed with Ramith chlorophorm extract gave the highest accelerated aging germination percentage (72%). However, the lowest viability of the traits were obtained from untreated seed with plant extracts. These results cleared that, these plant extracts had no adverse effects on seed viability or seedling

vigour. These results agreed with those reported by George and Patel (1992) they found that the active action of plant extracts for protecting seeds from storage pests without adverse effects on seed germination.

Table 3: Effect of clover varieties and plant material extracts on germination percentage, accelerated aging germination percentage, electrical conductivity, seedling length, seedlings dry weight and seedlings vigour index directly after harvest.

Characters	Germination percentage	Accelerated aging germination %	Electrical conductivity (us/cm/g)	Seedling length (cm)	Seedlings dry weight (g)	Seedling vigour index
Treatments						
A: Clover varieties						
Egyptian clover	96	71	0.165	10.5	0.051	4.9
Alfalfa	98	74	0.155	10.9	0.053	5.1
Fahl berseem	95	67	0.158	10.7	0.051	4.9
LSD at 5%	1.2	2.2	0.008	0.3	0.001	0.1
B: Plant material extracts						
Control	95	70	0.164	10.7	0.053	5.0
Ramith chlorophorm extract	96	72	0.155	10.6	0.051	4.9
Ramith ethanol extract	97	71	0.166	10.8	0.052	5.0
Oshar chlorophorm extract	97	71	0.160	10.6	0.051	4.9
Oshar ethanol extract	96	70	0.151	10.8	0.052	5.0
LSD at 5%	0.9	1.9	0.006	NS	0.001	NS

Data in (Table 4) showed that the variation among the studied clovers in seed germination, accelerated aging germination, electrical conductivity, insect infestation and seedling vigor traits and the effect of the studied plant materials on these traits, after six months from storage. Alfalfa seeds stored for six months had the highest in seed viability followed by Egyptian clover then Fahl berseem, while it gave the highest, germination percentage (91%), accelerated aging germination (65%), strongest seedlings (10.4 cm), seedlings dry weight (0.050g and seedling vigour index (4.6)) and this was disagreed with the data of insects inspection while the infestation level fall to (4.45%), compared with Fahl berseem (6.20%) and Egyptian clover (6.40%). From these results, the reduction rate in seed germination after six months in open air storage conditions reached (7% for Alfalfa and Egyptian clover and 9% for Fahl berseem), comparing with the unstored seeds. With respect to the effect of treating clover seeds with plant materials extracts, treated clover seed with Ramith chlorophorm extract produce the highest means of germination percentage and accelerated aging germination (93% and 67%, respectively) followed by Oshar ethanol extract (91% and 65%, respectively) Oshar chlorophorm extract (90 and 63%, respectively) and Ramith chlorophorm extract (87 and 61%, respectively).Seedlings vigour traits had the same trends, however, the treated seed have the strongest seedlings comparing with untreated seeds. The insect infestation level after six months in treated seed was less than untreated seed and the lowest level was (3.75%) in treated seed with Ramith chlorophorm extract followed by Oshar ethanol extract (4.33%).

Table 4: Effect of clover varieties and plant material extracts on germination percentage, accelerated aging germination percentage, electrical conductivity, insect infestation percentage, seedling length, seedlings dry weight and seedling vigour index, after storage with six months.

Treatments	Characters						
	Germination percentage	accelerated aging germination %	Electrical conductivity ($\mu\text{s/cm/g}$)	Insect infestation %	Seedling length (cm)	Seedlings dry weight (g)	Seedling vigour index
A: Clover varieties							
Egyptian clover	89	62	0.178	6.40	9.9	0.048	4.3
Alfalfa	91	65	0.162	4.45	10.4	0.050	4.6
Fahl Berseem	86	59	0.171	6.20	10.0	0.050	4.3
LSD at 5%	2.1	3.2	0.008	2.14	0.3	0.001	0.2
B: Plant material extracts							
Control	82	54	0.196	8.67	9.2	0.047	3.8
Ramith chlorophorm extract	93	67	0.145	3.75	10.6	0.051	4.7
Ramith ethanol extract	87	61	0.181	6.00	10.1	0.049	4.2
Oshar chlorophorm extract	90	63	0.168	5.66	10.2	0.050	4.5
Oshar ethanol extract	91	65	0.163	4.33	10.5	0.051	4.6
LSD at 5%	1.5	2.4	0.009	1.79	0.3	0.003	0.1

These results indicated the importance of protect the clover seed against storage insects. Also it was compound with Chaudhry (1992) and Khan and Siddiqui (1994) they reported the effectiveness of ramith and oshar extracts for pest protection (insects and microorganisms) also El-Rokh (2007) reported, Ramith chlorophorm was more active than Ramith ethanol extract.

Data presented in Table 5 show that after storage with 12 months, there were significant effects on the studied traits for the tested clovers. Alfalfa seed was the most tolerant for prolong the storage period for one year as measured with readings of germination percentage (85%), accelerated aging germination (52%), seedling length and seedling dry weight (0.050gm) comparing with Egyptian clover and Fahl berseem. Insect infestation levels was in opposite direction with seed quality, while increasing insect infestation decreased seed germination and seedling vigour traits, this was fair in Alfalfa while the level of insect infestation was (9.52 %) in the same time its germination percentage (85%) was the highest comparing with Fahl berseem, while its insect infestation level reach (10.80%) and its germination percentage was the lowest (72%).

After 12 months from store the treated clovers seed with plant extracts, there were significant effects on the studied traits, as illustrated from Table 5. Untreated clovers seed were the lowest in seed quality (as measured with seed germination (72%), accelerated aging germination (36%), seedling length (7.4 cm), seedling dry weight (0.044g) and seedlings vigour index (3.2)). Also were the highest in levels of insect infestation (16.33%) comparing treated seed with plant extracts.

Table 5: Effect of clover varieties and plant material extracts on germination percentage, accelerated aging germination percentage, electrical conductivity, insect infestation percentage, seedling length, seedlings dry weight and seedling vigour index after storage with 12 months..

Characters	Germination percentage	accelerated aging germination%	Electrical conductivity ($\mu\text{S/cm/g}$)	Insect infestation %	Seedling length (cm)	Seedlings dry weight (g)	Seedling vigour index
Treatments							
A: Clovers variety							
Egyptian clover	76	42	0.210	9.60	8.2	0.047	3.6
Alfalfa	85	52	0.183	9.52	8.9	0.050	4.3
Fahl berseem	72	39	0.229	10.80	8.5	0.048	3.5
LSD at 5%	1.9	2.6	0.001	2.33	0.4	0.001	0.2
B: Plant material extracts							
Control	72	36	0.233	16.33	7.4	0.044	3.2
Ramith chlorophorm extract	83	49	0.182	4.42	9.2	0.050	4.1
Ramith ethanol extract	75	44	0.218	11.00	8.4	0.048	3.6
Oshar chlorophorm extract	78	45	0.205	9.67	8.6	0.049	3.8
Oshar ethanol extract	80	47	0.200	6.33	9.1	0.050	4.0
LSD at 5%	1.5	2.2	0.001	1.94	0.3	0.002	0.1

Among the plant extracts, Ramith chlorophorm extract have more efficiency in reducing the insect infestation level while it not exceed (4.42%) followed by Oshar ethanol extract (6.33%) , Oshar chlorophorm extract (9.67%) and finally Ramith ethanol extract (11%).

Data presented in Table 6 show significant differences among the tested clovers in seed quality and insect infestation after 18 months from beginning storage. The importance of extend storage period for 18 months refers to if it possible to sowing the remainder clovers seed in the next year (as carry over seed), while the obtained data cleared that seed quality deterioration increased after 18 months from beginning storage especially in Fahl and Egyptian clover comparing with Alfalfa seed. Alfalfa seed germination percentage reached (72%) comparing with (62% and 54%) for both of Egyptian clover and Fahl berseem , respectively. These data were inherence with the increase in insect infestation levels while the infestation level in Alfalfa seed was the lowest (13.40%) followed by Egyptian clover (16.23%) and Fahl berseem (19.40%). Similar results were reported by Ramzan *et al.* (1990) and Srivastava and Pant (1989). They reported that Bruchids (*Callosobruchus* spp.) are important storage pests of legumes seed, and are known to cause considerable economic losses. Bruchid-damaged seeds do not germinate because of feeding on both of the endosperm and embryo.

Also Keals *et al.* (1997) reported that *Bruchidius trifolii* has been shown to reduce the seed carry over in trail plots by 50%. The former data cleared, the potentiality of sowing Alfalfa reminder seed in the next season if it stored in optimum conditions in open air meanwhile, Egyptian clover and Fahl berseem we can increase the seed quantity during sowing to redress seed quality deterioration.

Table 6: Effect of clover varieties and plant material extracts on germination percentage, accelerated aging germination percentage, electrical conductivity, insect infestation percentage, seedling length, seedlings dry weight seedlings vigour index, after storage with 18 months..

Characters	Germination percentage	accelerated aging germination%	Electrical conductivity (us/cm/g)	Insect infestation %	Seedling length (cm)	Seedlings dry weight (g)	Seedling vigour index
Treatments							
A:Clover varieties							
Egyptian clover	62	25	0.223	16.23	7.1	0.043	2.6
Alfalfa	72	31	0.200	13.40	7.6	0.047	3.4
Fahl berseem	54	22	0.240	19.40	7.4	0.044	2.4
LSD at 5%	4.2	3.7	0.006	2.19	0.4	0.002	0.2
B: Plant material extracts							
Control	60	23	0.252	22.67	6.7	0.043	2.6
Ramith chlorophorm extract	64	28	0.200	10.42	7.9	0.046	3.0
Ramith ethanol extract	63	25	0.227	17.67	7.2	0.044	2.7
Oshar chlorophorm extract	62	27	0.217	16.33	7.3	0.044	2.8
Oshar ethanol extract	64	28	0.209	13.00	7.8	0.046	3.0
LSD at 5%	3.5	2.8	0.004	1.82	0.4	0.002	0.2

With respect to plant extracts effect on seed quality and insect infestation levels, after 18 months from storage, significant effect was noticed as shown in (Table 6). In general, seed quality decreased meanwhile insect infestation increased with prolonging the storage period up to 18 months. The reduction was broad in untreated clover seed, while, germination percentage reduced to (60%), accelerated aging germination to (23%) also seedlings vigour decreased meanwhile insect infestation reached its highest levels (22.67%). Also, plant extracts lost its effectiveness while insect infestation increased gradually comparing with storage for six and twelve months to (10.42%, 13% and 17.67%) for clover seed treated with Ramith chlorophorm extract, Oshar ethanol extract and Ramith ethanol extract, respectively, and this was followed by the reduction in seed germination and seedlings vigour. The reduction in seed quality might be also due to the increase of some organic compounds consumption in respiration process with increasing storage period Malaker *et al.* (2008).

The correlation coefficient for the relationships between storage periods and germination percentage, accelerated aging germination percentage, insect infestation %, electrical conductivity and seedlings vigour index listed in (Table 7). There were negatively and highly significant correlations between the storage periods and germination% ($r=-0.900$), accelerated aging germination % ($r=-0.945$) and seedlings vigour index ($r=-0.887$). On the other hand, positively and highly correlation between the storage periods and both of insect infestation percentage ($r=0.855$) and electrical conductivity ($r=0.755$). Also, insect infestation percentage negatively and highly significant correlated with germination percentage ($r=-0.920$), accelerated aging germination ($r=-0.915$), seedlings vigour index ($r=-$

0.936) and highly positively correlated with electrical conductivity ($r=0.905$). This results agreed with those reported by Mersal *et al.* (2006)

Table 7: Correlation coefficient between storage periods and germination percentage, accelerated aging germination percentage, electrical conductivity, insect infestation percentage and seedlings vigour index.

	1	2	3	4	5
1-storage periods					
2- Germination %	-0.900**				
3- accelerated aging germination%	-0.945**	0.973**			
4-Insect infestation	0.855**	-0.920**	-0.915**		
5- Electrical conductivity	0.755**	-0.889**	-0.0880**	0.905**	
6- Seedlings vigour index	-0.887**	0.987**	0.967**	-0.936**	-0.900**

It was noticed that Egyptian clover (c.v (helaly), Alfalfa (c.v. Ismailia 1) and Fahl berseem seed differed in seed quality and insect infestation levels during storage in the open air for (6, 12 and 18 months) comparing with unstored seed. Alfalfa seed quality was most tolerant to extend storage periods until 12 months comparing with Egyptian clover and Fahl berseem as measured by (germination percentage, accelerated aging germination, electrical conductivity of leached seed and seedling vigour traits). Also the insect inspection for Alfalfa seed showed that the infestation was less than Egyptian clover and Fahl berseem due to differences in chemical components. If we store the remainder clover seed for the next year as (carry over seed) it should store in optimum conditions and we can increase seeding rate to compensate seed quality reduction, especially in Egyptian clover and Fahl berseem, while seed germination reduced less than the standard level for certified seed (the minimum germination percentage for certified clover seed should not be less than 85%). Clover seed can be protected from storage pests for 12 months especially (storage insects) with treating seed before storage by plant extracts. Treating clover seed with Ramith chlorophorm extract and Oshar ethanol extract were more effective for protect clover seed from insects comparing untreated seeds without harmful effects on seed quality. Also, with increasing insect infestation seed germination and seedling vigour decreased .

REFERENCES

- Abbassi, K. ; Z. Kadiri and S. Ghaout (2003) . Biological effects of alkaloids extracted from three plants of Moroccan arid areas on the desert locust. *Physiological Entomology*, 28: 232–236.
- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
- Abd El-Wahab, H. A. (2003). Efficiency of leaves extracts of castor bean plant against *Aphis gossypii* (Glover) and *Tetranychus urticae koch* .on cucumber plant. *J. Agri. Sci. Mansoura Univ.*,28 (5):4029-4038.
- Abo-El-Ghar, G. E. S. ;G. I. Zohdi; A. I. Farag and A. E. Snad (1990). Effect of some plant extracts on the development reproduction of the spider mite *tetranychus urticae koch* and stigmatid predator *Agistemus exsertus* Gonzales-Bull-Ent-Soc- Egypt, Econ-Ser., 18: 105-116.

- Ajabnoor, M.A.; M. A. Al- Yahya ;M. Tariq and A. A. Jayyab (1984). Antidiabetic activity of *Hammada salicornicum*. Fitoterapia, LV, 107-109.
- Akinloye, A. K. ; M.O. A. Abatan and B. O. Oke (2002). Histomorphometric and histopathological studies on the effect of *Calotropis procera* (Giant milkweed) on the male reproductive organs of wistar rates. African J. Biomedical Res., 5(1-2): 57-61.
- Al-Shanawani, M. A. A. (1996). Plant used in Saudi folk medicine. King Abdul-Aziz City for Science and Technology (KACST), Riyadh, p.162.
- Chaudhry, M. I. (1992). Efficacy of botanical pesticides against *Plecopter reflexa* Guen. (Noctuidae, Lepidoptera), shisham defoliator. Pakistan J. Forestry, 42(4): 199-202.
- Elias, S. ; A. Garay and E. Gatch (2009). Seed Quality Testing and Certification : Resources useful in organic seed production. Organic Seed Resource Guide. Article January 22,2009.
- El-Lakwah, F. A. ; M. S. Hamed and A. R. Mohamed (1999). Effect of diet treatment with African chillifruits (*capsicum frutescens*) powder on populations of (*Sitophilus oryzae* L.,) and *Tribolium castaneum* (Herbst). Zagazig J. Agric. Res., 26 (1): 199-213.
- El-Rokh, A. R. (2007). Chemical studies on some natural extracts and their constituents to control some Aphid species. M.Sc. Thesis, Fac. of Sci., Mansoura Univ. Egypt.
- Evans, W.C. (2001). Trease and Evans Pharamacognosy. Vol.15, W.B. Saunders Company Ltd.,London, Philadelphia, Toronto, Sydney, Tokyo, p22.
- Finney, D. J.(1952). Probit analysis statistical treatment of the sigmoid response curve. Cambridge Univ. press, pp 318.
- Freedman, B. ; J. Noak and W. F. Kwolek (1979). Abioassay forplant derived pest control agent using the European cornborer.J. Entomol., 72:45-54.
- George, G. and Patel,R. J. (1992). Mint, *Mentha spicata* a promising botanical for green gram against pulse beetle, *Callosobruchus analis* F. Indian J. of Plant Protection 20(1):66-69.
- Gomez, K. A. and A. A. Gomez (1984). Statistical procedures for agricultural research. 2nd Ed. John Whley & Sons.
- I. S. T. A. (1985). International Rules for Seed Testing, Seed Science and Technol., 13 (2): 421-463.
- Jood, S. ; A. C. Kapoor and S. Ram (1996). Evaluation of some plant products against *Trigoderma granarium* Evarts in sorghum and their effects on nutritional composition and oranoleptic characteristics .J. Stored Prod. Res., 32 (24): 345-352.
- Keals, N. ; D. Hardie and R. Emery (1997). Bruchids- secret and eaters. Western Australian year Book (1997), Western Australian Government Dress.
- Khan, S. M. and M. N. Siddiqui (1994). Potential of some indigenous plants as pesticides against the larvae of cabbage butterfly *Pieris brassicae* L. Sarhad J. Agric., 10(3) :291-301.
- Krishnasamy, V. and D. V. Seshu (1990). Phosphine fumigation influence on rice seed germination and vigor. Crop Sci. 30 :28-35.

- Li, X.; D. Su and Q. Yuanu (2007). Ridge-Furrow planting alfalfa(*Medicago sativa L.*) for improved rainwinter harvest in rainfed areas in Northwest China. *Soil and Tillage Res.*, 93: 117-125.
- Mabberley, D.J. (1997). *The Plant –Book, Aportable dictionary of vascular plants.* Cambridge University Press, UK, P.326.
- Malaker, P. k.; I. H. Mian; K. A. Bhuiyan; A. M. Akanda and M. M. A. Reza (2008). Effect of storage containers and time on seed quality of wheat. *Bangladesh J. Agric. Res.*, 33 (3): 469-477.
- Matthews, S. and A. P. Alison (1987). *Electrical conductivity test methods (2nd edition)*, 37-43. published by ISTA.
- Mehrotra, K. N. (1993). Status of insecticide resistance in insect pests. *Indian Scenario.* In Dhaliwal, G. S. and Singh, B. (Eds). *Common Wealth Publishers, New Delhi* 30-50.
- Mersal, I. F. ; Sh. A. Abou El-Goud ;T. K. Abd El-Aziz and Iman, A. I. Mohamed (2009). Effect of spraying plant extracts on seed quality and insect infestation of some cowpea cultivars. *J. Mansoura* 34 (3): 1839-1851.
- Mersal, I.F. ;A. A. M. El-Emam and Amal, H. Selim (2006). Effect of storage period, seed moisture content and insecticides treatment on wheat (*Triticum aestivum,L.*) seed quality.*Annals of Agric.Sci., Moshtohor*, 44(1): 111-124.
- Meshram, P. B. (2000). Antifeedant and insecticidal activity of some medicinal plant extracts against *Plecoptera reflexa* Gue. (Lepidoptera : Noctuidae). *Indian Forester*, 126 (9): 961-965.
- Pacheco, I. A.; M.F. De Castre; D. De Plaua; A. L. lourencao; S. Bolonhezi and M. K. Barbieri (1995). Effecienfy of soybean and castor oils in control of *Callosobruchus maculates* (F.) and *Callosobruchus phaseoli* (Gyllenhal) in stored chick peas (*Cicer arietinum L.*). *J. of Stored Prod. Res.*, 31 (3) 221-228.
- Pooenheimer, H. R. (1959). The Origin of the Egyptian clover with critical revision of some closely related species. *Bull. Res. Coun.*, 1se. 7D: 202-221.(C. F. Computer Search).
- Ramzan, M. ; B.S. Chahal and B. K. Judge (1990). Storage losses to some commonly used pulses caused by pulse beetle (*Callosobruchus maculates*). *Journal of Insect Sci.*, 3: 106–108.
- Salama, H.S. and I. A. Ismail (2005). Potential of certain natural extracts the control of the red palm weevil, *Rhynchophorus ferriegineus* (Oliver). *Archives of Phytopathology and Plant Protection.* 40(4) :233-236
- Sathiyamoorthy, P.; H. Lugasi-Evgi ; P. Van Damme ; A. Abu-Rabia ; J. Gopas ; Y. Pollack and A. Golan- Goldhirsh (1997). Larvicidal activity in desert plants of negev and bedouin market plant products. *International J. of Pharmacognosy*, 35: 265-273.
- Srivastava, K.M. and J.C. Pant (1989). Growth and developmental response of *Callosobruchus maculatus* (Fabr.) to different pulses. *Indian J. of Entomology*, 51: 269–272.
- Tackholm, V. (1956). *Students Flora of Egypt.* Published by Anglo. Egyptian Book. Shop, Cairo.

- Tembo, E. and R.F. A. Murfih (1995). Effect of combining vegetable oil with pirimiphos methyl for protection of stored wheat against Sitophilus grainius (L.). J. of Stored Prod. Res., 31 (1):77-81.
- Vijay,D. ; M. Dadlani and S. Nagarajan (2009). Role of sorption properties and water status in control of seed longevity patterns. Current Sci., 96(8): 1103- 1109.
- Zedan, H. A. ; E. M. Anwar and M. A. El-Gindy (2007). Effect of some plant extracts and its binary mixtures with chlorpyrifos against ZND instar larvae of cotton leaf worm spodoptera Li Horalis (BOISD) under laboratory conditions.J. Agric. Sci. Mansoura Univ., 32 (6): 4821-4828.

تأثير المستخلصات النباتية على جودة تقاوى بعض محاصيل العلف أثناء التخزين عبير الوردي أحمد إبراهيم , إبراهيم فتحي مرسل و مجدي إبراهيم العبادي . قسم بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية

تهدف الدراسة الى تحديد التأثير الحيوي للمستخلصات النباتية لكلا من (نبات الرمث ونبات العشار) على سوسة البرسيم, وذلك بمعاملة بذور البرسيم المصري (صنف هلالى) و البرسيم الحجازي (صنف إسماعيليه ١) و البرسيم الفحل بهذه المستخلصات ومعرفة تأثيرها على جودة التقاوى والأصباية الحشرية وذلك عند تخزين التقاوى لفترات مختلفة (٠، ٦، ١٢، ١٨ شهرا). أجريت تجربة معملية بمعمل بحوث تكنولوجيا البذور بالمنصورة- قسم بحوث تكنولوجيا البذور-معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية خلال عامي ٢٠٠٨ و ٢٠٠٩ م وأوضحت النتائج أن التأثير الحيوي للمستخلص الكلوروفورمى لنبات الرمث و الإيثانولى لنبات العشار الأكثر فعالية على سوسة البرسيم مقارنة بالمستخلص الكلوروفورمى للعشار و الإيثانولى للرمث وكانت قيم Lc50 (٤٧٥٣,٩ - ٨٥١٤,٣ - ١١٠٢٦,٩ - ٢٠٦٤٩,٦ جزء في المليون) على التوالي.

وكانت نسبة الإنبات بصفه عامه بعد التخزين لمدة ٦ شهور أعلى من الحد الأدنى لمستويات القبول للتقاوى المعتمده (٨٥%) حيث بلغت (٩١%) فى بذور البرسيم الحجازي تلاها البرسيم المصري (٨٩%) ثم الفحل (٨٦%). كما أظهرت نتائج الفحص الحشري أن بذور البرسيم الحجازي كانت الأقل إصابة (٤,٤٥%) تلاها البرسيم المصري والفحل (٦,٤ و ٦,٢%). كما أعطت البذور المعامله بمستخلص الرمث الكلوروفورمى أعلى نسبة إنبات ووقوه بادرات وكانت الأقل فى نسبة الإصابة الحشرية (٣,٧٥%) مقارنة بالبذور الغير معاملة حيث إنخفضت نسبة الإنبات لمستوى أقل من الحد الأدنى للقبول للتقاوى المعتمده (٨٢%) وارتفعت الإصابة الحشرية بها إلى (٨,٦٧%).

بعد التخزين لمدة ١٨ شهر إنخفضت نسبة الإنبات لمستوى أقل من الحد الأدنى لمستويات القبول للتقاوى المعتمده فى جميع الأصناف و بذور البرسيم الحجازي الأقل إنخفاضا تلاها البرسيم المصري ثم الفحل وكذلك إنخفضت قوه البادرات الناتجه مقارنة بفترات التخزين السابقه , كما إرتفعت نسبة الإصابة الحشرية بالبذور الغير معاملة الى (٢٢,٦٧%) مقارنة بالبذور المعاملة بالمستخلص الكلوروفورمى للرمث (١٠,٤٢%).

وجد ارتباط معنوي سالب بين فترات التخزين والنسبة المئوية للإنبات والإنبات بعد إختبار الشبخوخة ودليل قوه الإنبات كما وجد ارتباط معنوي موجب مع كلا من النسبة المئوية للإصابة الحشرية والتوصيل الكهربى للبذور . كما وجد أيضا ارتباط معنوي سالب للإصابة الحشرية والنسبة المئوية للإنبات ودليل قوه الإنبات.

توصى هذه الدراسة بإمكانية معاملة بذور (البرسيم المصري (صنف هلالى) و البرسيم الحجازي (صنف إسماعيليه ١) و البرسيم الفحل) قبل التخزين بالمستخلص الكلوروفورمى للرمث أو الإيثانولى للعشار للوقاية من الإصابة بسوسة البرسيم. وزراعة التقاوى المعتمده فى نفس موسم إنتاجها وذلك للحصول على تقاوى عاليه الجودة وإذا خزنت تقاوى البرسيم (البرسيم المصري و البرسيم الحجازي (صنف إسماعيليه ١) و البرسيم الفحل) للعام التالي فيجب زيادة كميته التقاوى عند الزراعة لتعويض التدهور فى جودة التقاوى.

قام بتحكيم البحث

أ. د/ أحمد نادر السيد عطية

أ. د/ محمود إبراهيم العميرى

كلية الزراعة – جامعة المنصورة

مركز البحوث الزراعية