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Physiological and biochemical evaluation of cowpea seeds (*vigna unguiculata* L.) Storability using seed coating technique

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This study aimed to investigate the response of *Vigna unguiculata* L. dry seeds to chitosan, 18-18-18 fertilizer, thiamethoxam, mefenoxam and fludioxonil. These different seed treatments were used in combinations to improve seed quality by (I) reducing the deterioration resulting from seed storage conditions, (II) combating of cowpea weevil (*Callosobruchus maculatus*) in the store and fungi at sowing date of seeds. Data were recorded for the characteristics of seed germination. All treatments were compared with untreated seeds. Seeds were stored for 12 months and examined after 4, 8 and 12 months. In a separate experiment, the seeds were exposed to contact with the three fungi soil in the laboratory. Treatment (5): (Chitosan) + (18-18-18 fertilizer) + (thiamethoxam + mefenoxam + fludioxonil (CruiserMaxx)) + (fludioxonil (Maxim)) recorded the best values for the most studied characteristics. Seed production companies may be recommended to apply treatment (5) to produce high-quality cowpea seeds and free from the insect injury and characterized by tolerance of soil fungal infection at field seed sowing.

Keywords: cowpea seeds, weevil, chitosan, thiamethoxam, mefenoxam, fludioxonil.

INTRODUCTION

Cowpea is cultivated in the tropics for its edible tender long pods and dry seeds. It is also called Bombay cowpea or catjang. Immature pods and fresh seeds are cooked as a vegetable or canned. Green foliage is fed to the cattle. Cowpea is rich in protein, carbohydrates, oils, calcium, phosphorus and vitamin A (Doijode, 2001). The world cowpea planting area was 58.1 million hectares and the Production was 33.5 million tones. Africa has been responsible for 95.8% of worldwide cowpea production (FAO Stat 2017). Cowpea (*Vigna unguiculata L. Walp*) is attacked by pests both in the field and during storage. The major storage pest of cowpea is *Callosobruchus maculatus Walp*. Pods of cowpea stored for 8 months could have as much as 50% of the grains damaged by *C. maculatus* (Caswell, 1984). *Callosobruchus maculatus* is a cosmopolitan field-to-store pest ranked as the principal postharvest pest of cowpea in the tropics and subtropics of the world (Bamphitihi et al., 2014). Damage caused by cowpea weevils in seeds affected germination (Melo et al., 2010). This pest causes substantial quantitative and qualitative losses evident by seed perforation or holes and reduction in weight, market value and germination ability of seeds (Oluwafemi, 2012). *Callosobruchus maculatus* infestation significantly damaged seeds of *Vigna unguiculata* resulting in reduced seed germination and altered nutrient quality of stored beans (Mofunanya and Namgbe, 2016). Infestation of *Phaseolus lunatus* by the cowpea weevil, *Acanthoscelides obtectus* reduced seed germination with changes in the biochemical composition of seeds (Mofunanya, 2017). This damage caused by storage pests is threatening as global population growth is estimated to 9 billion persons inhabiting the Earth by 2050; a 60% increase in food production must be attained. Thiamethoxam is one of the insecticides used for seed treatment. This is a systemic neonicotinoid product that, besides its insecticide function, has bioactivator properties. This means that thiamethoxam is an organic substance that modifies plant growth, has the potential to interfere with DNA transcription, gene expression and affect membrane proteins, metabolic enzymes and mineral nutrition (Castro and Pereira, 2008). Aluminum phosphide is used as a fumigant to protect stored grain from insects and rodents. In the presence of moisture, aluminum phosphide releases phosphine, which is highly toxic. The fatality rate was 59% among 195 patients admitted to a hospital in Northwest India. The fatal dose was 1.5 g, and the predominant clinical feature was hypotension (Singh et al., 1996). Thiamethoxam acts on the expression of genes that synthesize and activate enzymes related to plant growth, increasing the production of plant hormone precursor amino acids (Castro, 2006). Furthermore, (Cataneo, 2008) reported that this insecticide increases the expression of seed vigor, dry matter accumulation, photosynthetic rate and root depth. (Castellanos et al., 2017) found that treatment of bean seeds with thiamethoxam increased plants chlorophyll content, and Seed treatment with thiamethoxam was found to be associated with increase in vigor (Almeida et al., 2009). Initially, fungicides were developed for the protection of crops against diseases and pests. (Bierman et al., 2006) this is performed by seed companies devoted to ensure that seed of high quality is delivered. The fungicides Celest® XL (fludioxonil and mefenoxam), Apron® XL (mefenoxam) and Apron® Star 42 WS (thiamethoxam, metalaxyl-m and difenoconazole) were produced by Syngenta for their low risk to the environment. The effect of these fungicides on the germination and vigor of maize seeds is largely unknown. Aim of the work: improving seed quality by 1: reducing the deterioration resulting from seed storage conditions, 2: combating of cowpea weevil (*Callosobruchus maculatus*) in the store and soil fungi at field seeds sowing.

MATERIALS AND METHODS

The current study was conducted during the years of 2015 and 2016. One cowpea cultivar (cv Teba) was used in an experimental trial to reduce the deterioration resulting from seed storage conditions and combating of cowpea weevil (*Callosobruchus maculatus*) in the store and fungi at sowing date of seeds. This evaluation trial was achieved in a laboratory at Vegetables Research Departments, Dokki-Giza. The intact dry seed yield of cowpea has been obtained from plants grown in Kaha Vegetables Research Station, Kalubia Governorate. Following the recommendation of the agricultural pesticides committee–Ministry of agriculture and land reclamation–Egypt concerning the integrated pest management program on cowpea that could lead to the combating the fungi and cowpea weevil in the field.

Experimental details

Experiment-I:

Effect of seed coating with polymer, fertilizer, fungicide and insecticide on seed quality of cowpea during storage.

Treatment details:

Treatment (1):

(Chitosan) Three percentage of chitosan coating polymer was prepared by adding 3 g chitosan (Egyptian Petroleum Research Institute) into 100 ml of 1% v/v acetic acid. Then, the chitosan was sprayed and mixed well into 500 g seeds. The seed moisture content (MC) of 10±2% was obtained after drying at 35ºC.

Treatment (2):

(Chitosan) +(18-18-18 fertilizer). Basically, a compound fertilizer is one that has several elements included or mixed like Nitrogen (N), Phosphorus (P), Potassium (K), boron, copper, cobalt and many other elements that enhance the growing characteristics of a plant. 1000 ppm compound fertilizer per 500 g seeds was prepared by adding 1.85 g fertilizer into chitosan solution.

Treatment (3):

(Chitosan) + (thiamethoxam + mefenoxam + fludioxonil (Cruiser Maxx). The CruiserMaxx 420 FS formulation used in the tests contained thiamethoxam (22.61% ai - insecticide), mefenoxam (1.70% ai - fungicide) and fludioxonil (1.12% ai - fungicide) and was obtained from Syngenta – Egypt. Adding 0.75 ml of Cruiser

Maxx into the composition of the chitosan solution. Then, the chitosan was sprayed and mixed well with 500 g seeds.

Treatment (4):

(Chitosan) +(18-18-18 fertilizer) + (thiamethoxam + mefenoxam + fludioxonil (CruiserMaxx).

Treatment (5):

(Chitosan) +(18-18-18 fertilizer) + (thiamethoxam + mefenoxam + fludioxonil (CruiserMaxx)) + (fludioxonil (Maxim)). The Maxim 420 FS formulation used in the tests contained fludioxonil (40.3% ai – fungicide effect) and was obtained from Syngenta–Egypt. Adding 0.325 ml of Maxim into the composition of the chitosan and the other components of solution .Then, the chitosan was sprayed and mixed well with 500 g seeds.

Untreated seeds (uncoated seed):

Seeds were divided into two parts: part 1, directly after seed harvesting from the field, the treated and untreated seeds were exposed to insect infection by cowpea weevil in cages covered by gauze. Seeds were stored at normal storage conditions. Exposure period was three months at room temperature conditions, in the end of the experiment, beetles were removed and assess damage was recorded. Five categories were used: 1= No damage, 2 = 1 - 10% damage, 3 = 11 - 30% damage, 4 = 31 - 60% damage, 5 = > 60% damage (Ekbom and Müller, 2011). Data were recorded for the characteristics germination percentage, germination rate, weight of 1000 seeds, shoot length, root length, seedling length, fresh and dry weights. All treatments were compared with untreated seeds. Part 2 of seed samples: the treated and untreated seeds were kept in sterilized conditions against insect infestation. Data were recorded for the characteristics minerals content, isoenzymes activity, protein content, total free amino acids and total soluble sugars. Seeds were stored for 12 months and examined after 4, 8 and 12 months.

Experiment-II:

The samples of seeds were incubated on petri plates containing potato dextrose agar medium to contact with the three soil fungi, *Fusarium oxysporum, Fusarium solani and Rhizoctonia solani* in the laboratory. Incubation period was seven days at 28ºC. These isolates were tested for pathogenicity in laboratory at Seed Pathology Research - Plant Pathology Research Institute, Egypt. The disease severity assessed based on 0-100% scale: 0 - 25% = tolerant, 26 - 50% moderately tolerant, 52 - 75% = moderately susceptible and 76 - 100% susceptible, scale was adapted from Mukankusi et al., (2010).

Germination tests:

Germination tests were carried out on three replicates of 25 seeds. The seeds were set to germinate in between moistened paper towels. Seeds were kept at 25ºC°±2 for 10 days. The numbers of seeds were counted daily up to 7 days.

Germination percentage:

Germination percentage (GP) was calculated as following:

$$GP = \frac{\text{Total number of germinated seeds}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Mean number of days required for germination} = \frac{(G1 \times N1) + (G2 \times N2) + \text{-----} + (Gn \times Nn)}{G1 + G2 + \text{------} + Gn}$$

Germination rate:

Germination speed (GS) or germination rate (GR) was calculated according to Edmond and Drapala (1958) as: Where: G = number of germinated seeds in a certain day, n = number of this certain day. At least 10 randomly chosen seedlings (control) and each other treatments were taken for measurements of different growth criteria; namely shoot length (cm), root length (cm), seedling length, fresh and dry weights (g).

Chemical composition of cowpea seedlings:

Determination of isoenzymes content of cowpea seedlings:

Antioxidant isozymes activity in native page Electrophoresis conducted to identify isozyme variations among cowpea seed coating treatments using two isozyme systems: 1- Polyphenyl oxidase isoenzymes (EC. 1.10.3.1) leaves samples (100 mg fresh weight) were determined by the method of Thipyapong et al., (1995) and Bradford (1976). 2- Peroxidase isoenzymes (EC. 1.11.1.7) pod samples were determined by the procedure described by Ros Barcelo et al., (1987).

Determination of minerals content:

Nitrogen content was determined using the dried parts by micro-Kjeldahl method accorded to

Markaham (1942) using boric acid modification as described by Ma and Zuazage (1942). Phosphorus content (g /100 g dry weight) was determined calorimetrically according to the method of Jackson (1958). Potassium contents (g/100g dry weight) were determined against a standard using flame-photometer (Piper, 1950).

Determination of protein content (g/100g dry weight):

Crude protein content was calculated by multiplying total nitrogen by 6.25**,** A.O.A.C. (1990).

Determination of total free amino acids (g/100g dried weight):

According to (Yemm and Cocking, 1955).

Determination of total soluble sugars (%):

Total soluble Sugars were extracted according to Ackerson (1981)

Statistical analysis:

The results were analyzed using Minitab statistical package version-15; one way ANOVA–completely randomized analysis (combined analysis over 2 years) was performed on all treatments at each period of storage data. Also, data were tested for least significant differences 5% (Snedecor and Cochran, 1989) to compare the average of the determined parameters.

RESULTS AND DISCUSSION

Physiological analysis of cowpea seeds:

Table (1) shows the mean performances for the characteristics seeds categories affected by cowpea weevil, 1000 seed weight, germination percentage, germination rate, fresh weight of seedling, dry weight of seedling, dry matter ratio, Shoot length of seedling , root length of seedling and seedling length. The effect of seed coating treatments and storage periods on these characteristics was studied and The results showed that the treatments (3), (4) and (5) had not been affected by the infection of the insect (Category of seeds affected by cowpea weevil = 1), while the treatment (2) was relatively affected (Category of seeds affected by cowpea weevil = 3). On the other hand, control and Treatment (1) had been severely affected by the insect (Category of seeds affected by cowpea weevil= 5). Treatment (2) was the least affected by the storage period despite the insect damage, the reason of the effect of nutrients on the insect without the use of insecticides may be the different dietary behavior of insects; which may lead to toxicity or prevent feeding. These results were in line with Ibanez et al., (2017) who mentioned that according to the growth rate hypothesis, P-rich insects had higher growth rates. It is therefore expected that P-rich insects will prefer plants that are high in protein (hence in nitrogen, N) and phosphorus (P), but the reasons why different species have contrasting intake targets remain unclear. It is clear that treatments (3), (4) and (5) were the best values in all characteristics and in all seed storage periods; considering, that it was not affected by the insect injury. These results were harmony with Hameed et al., (2014) that indicated the final germination percentage was significantly increased after chitosan priming. Also, Sadeghi et al., *(*2011) suggested that there was possibility that similar germination responsive genes may be activated because of chitosan priming under osmotic stress. These results are in agreement with Tamindžić et al., (2016) who reported that all seed treatments resulted in significantly higher shoot fresh and dry weights than control, excepting the treatment with Maxim XL-035 FS + Cruiser 350-FS on shoot dry weight. Fresh root weight was significantly increased in Maxim XL-035 FS and Maxim XL-035 FS + Cruiser 350-FS treatments but significantly decreased in Maxim XL-035 FS + Gaucho 600-FS treatment. While, root dry weight significantly decreased after treatments with Maxim XL-035 FS + Gaucho 600-FS and Maxim XL-035 FS + Cruiser 350-FS compared to control. Also, Castro and Pereira (2008) who had found that thiamethoxam had the capability of inducing physiological changes in plant. It can increase mineral nutrition of the plant, which promotes positive responses in plant development and productivity. It also resulted in higher enzymatic activity caused by thiamethoxam, which increased both primary and secondary metabolism, due to the fact that some treatments such as thiamethoxam might have an activating effect, thus increasing the size of roots and shoots. These results are in agreement with Almeida *et al*. (2013) who reported that shoot length in treatment with Maxim XL-035 FS + Cruiser 350- FS was significantly decrease compared to other treatments and control. But these results are disagreement with Kuhar et al., (2002) that stated greatest shoot length in treatment with Maxim XL-035 FS + Cruiser 350-FS and it was significantly greater than in treatments with Maxim XL-035 FS and Maxim XL-035 FS + Gaucho 600-FS.

Table (1): Effect of seed coating treatments and seed storage periods on germination percentage, germination rate (day), 1000 seed weight (g) and seedling characteristics of cowpea seeds.

Seed storage periods	Seed coating treatments	Categories of seeds affected by cowpea weevil	1000 seed weight (g)	Germination Percentage (%)	Germinationrate (day)	Fresh weight of seedling (g)	Dry weight of seedling (g)	Dry matter ratio	Shoot length of seedling (cm)	Root Length of seedling (cm)	Seedling length (cm)
	Before storage and treatments	1	198.86	99.8	4.47	1.44	0.120	0.08	28.08	7.18	35.26
4 months	**Control**	5	161.06 ^{d}	0.00 ^{c}	0.00 ^{b}	0.00 ^{c}	0.00 ^{c}	0.00 ^{d}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	Treatment 1	5	163.37 ^{d}	0.00 ^{c}	0.00 ^{b}	0.00 ^{c}	0.00 ^{c}	0.00 ^{d}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	Treatment 2	3	202.28 ^{a}	98.16 ^{a}	4.61 ^{a}	1.43 ^{b}	0.12 ^{a}	0.09 ^{a}	21.53 ^{b}	7.56 ^{a}	29.10 ^{b}
	Treatment 3	1	199.58 ^{ab}	94.33 ^{b}	4.51 ^{a}	1.48 ^{a}	0.11 ^{b}	0.07 ^{c}	20.65 ^{c}	5.85 ^{d}	26.51 ^{c}
	Treatment 4	1	196.54 ^{bc}	93.16 ^{b}	4.44 ^{a}	1.42 ^{b}	0.11 ^{b}	0.08 ^{b}	25.37 ^{a}	6.33 ^{b}	31.70 ^{a}
	Treatment 5	1	194.16 ^{c}	93.83 ^{b}	4.56 ^{a}	1.42 ^{b}	0.12 ^{b}	0.08 ^{b}	18.38 ^{d}	6.16 ^{c}	24.54 ^{d}
	LSD 0.05	-	2.38	2.12	0.23	0.029	0.01	0.001	0.33	0.055	0.33
8 months	**Control**	5	137.14 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{e}	0.00 ^{d}	0.00 ^{e}
	Treatment 1	5	135.83 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{e}	0.00 ^{d}	0.00 ^{e}
	Treatment 2	3	199.67 ^{a}	95.00 ^{ab}	4.58 ^{ab}	1.38 ^{ab}	0.12 ^{a}	0.08 ^{a}	20.80 ^{b}	6.13 ^{b}	26.93 ^{b}
	Treatment 3	1	197.54 ^{ab}	95.83 ^{a}	4.71 ^{a}	1.40 ^{a}	0.11 ^{b}	0.07 ^{b}	19.18 ^{c}	5.74 ^{c}	24.93 ^{c}
	Treatment 4	1	194.47 ^{bc}	92.83 ^{b}	4.42 ^{b}	1.41 ^{a}	0.11 ^{ab}	0.08 ^{ab}	21.52 ^{a}	6.25 ^{a}	27.78 ^{a}
	Treatment 5	1	192.74 ^{c}	94.83 ^{ab}	4.55 ^{ab}	1.34 ^{b}	0.11 ^{ab}	0.08 ^{a}	17.33 ^{d}	6.13 ^{b}	23.46 ^{d}
	LSD 0.05	-	4.10	2.54	0.16	0.048	0.01	0.001	0.43	0.043	0.43
12 months	**Control**	5	116.54 ^{e}	0.00 ^{d}	0.00 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{b}	0.00 ^{e}	0.00 ^{d}	0.00 ^{e}
	Treatment 1	5	118.82 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{b}	0.00 ^{e}	0.00 ^{d}	0.00 ^{e}
	Treatment 2	3	197.4 ^{a}	93.33 ^{a}	4.65 ^{a}	1.38 ^{a}	0.11 ^{ab}	0.08 ^{a}	18.74 ^{b}	5.82 ^{b}	24.56 ^{b}
	Treatment 3	1	195.02 ^{b}	92.33 ^{ab}	4.57 ab	1.37 ^{ab}	0.12 ^{a}	0.09 ^{a}	17.84 ^{c}	6.17 ^{a}	24.02 ^{c}
	Treatment 4	1	192.80 ^{c}	89.00 ^{bc}	4.39 ^{bc}	1.34 ^{b}	0.11 ^{b}	0.08 ^{a}	20.15 ^{a}	6.16 ^{a}	26.32 ^{a}
	Treatment 5	1	192.15 ^{c}	87.00 ^{c}	4.33 ^{c}	1.35 ^{b}	0.12 ^{a}	0.09 ^{a}	15.44 ^{d}	5.31 ^{c}	20.76 ^{d}
	LSD 0.05	-	1.73	3.71	0.20	0.029	0.01	0.011	0.48	0.054	0.49

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

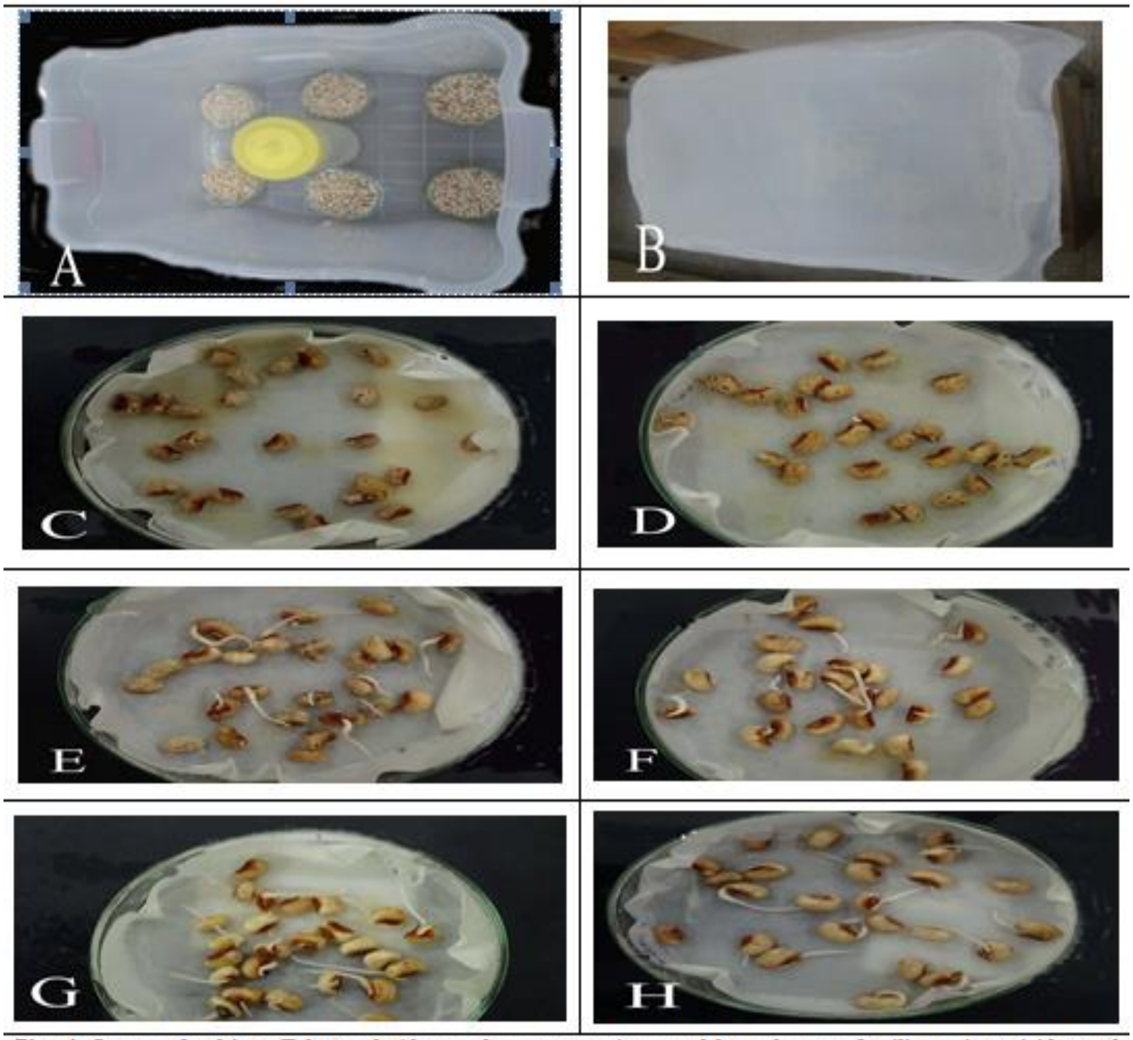

Fig. 1. Image of cultivar Teba seeds (dry seeds cowpea cv.) treated by polymers, fertilizers, insecticides and fungicides as seed coating, with weevil damage, Cages intended for infection by Cowpea seed weevil (A) and (B), control with ranking 5, resulting in dead seed (C), the treatment 1 with ranking 5, resulting in dead seed (D), the treatment 2 with ranking 3, resulting in normal seedling (E), the treatment 3 with ranking 1, resulting in normal seedling (F), the treatment 4 with ranking 1, resulting in normal seedling (G) and the treatment 5 with ranking 1, resulting in normal seedling (H).

Generally, all seed treatments increased root length compared to control. It had been assessed that the treatment with thiamethoxam produces plants with greater root growth and higher growth.

Chemical composition of cowpea seedlings:

Determination of isozymes:

Peroxidase in the first seed storage period:

Peroxidase electrophoretic patterns in Table (2) exhibit maximum of very well visible 4 consisted bands on the gel that tightly associated with relative mobility (Rf) ranged from 0.094 to 0.840. Bands characterized by clear inter and intra polymorphism in presence and absence. Variable densities among different treatments, the treatment (5) produced 4 bands with Rf 0.094, 0.335, 0.510 and 0.840. different densities whereas the bands at Rf 0.094 had higher density

Peroxidase isozyme in the third seed storage period:

Peroxidase electrophoretic patterns in Table (3) showed maximum of very well visible 5 consisted bands on the gel that tightly associated with relative mobility ranged from 0.082 to 0.769. The treatment (5) produced 5 bands with relative mobility 0.082, 0.293, 0.544, 0.658 and 0.769.

Table (2): Peroxidase isozymes ideogram analysis of cowpea seedlings in the first seed storage period.

Rf	Control	T1	T2	T3	T4	T5
0.094	++	+	+	++	++	+++
0.335	+	+	—	++	++	++
0.510	—	—	—	+	—	++
0.840	+	+	+	+	+	++

-: absent, +: low density, ++: moderate density and +++: high density

Table (3): Peroxidase isozymes ideogram analysis of cowpea seed coating treatments in the third seed storage period.

Rf	Control	T1	T2	T3	T4	T5
0.082	++	+	+	++	+	++
0.293	+	+	+	+	+	+
0.544	+	+	+	+	+	+
0.658	—	—	—	+	+	++
0.769	+	—	—	++	+	++

-: absent, +: low density, ++: moderate density and +++ : high density

These results are in agreement with those of Thobunluepop (2009) who reported similar data when rice seeds coated by traditional fungicide, biological fungicide polymers performed the best of seed vigor because they could maintain the antioxidative scavenging enzymes are ascorbate peroxidase (APX) and Superoxide dismutase (SOD) and a high antioxidant activity.

Polyphenyl oxidase electrophoresis in the first seed storage period:

Polyphenyl oxidase electrophoretic patterns in Table (4) produced a maximum of very well visible 11 consisted bands very well visible on the gel which tightly associated with relative mobility range from 0.041 to 0.919 that characterized by clear inter and intra polymorphism in bands presence and absence and variable densities among the different treatments. The treatments (4 and 5) got the higher isozyme expression that produced 9 bands with Rf 0.041, 0.123, 0.297, 0.416, 0.626, 0.685, 0.743, 0.834 and 0.919

Polyphenyl oxidase electrophoresis in the third seed storage period:

Polyphenyl oxidase electrophoretic patterns in Table (5) showed maximum of very well visible 6 consisted bands on the gel which were tightly associated with Rf ranged from 0.459 to 0.961. The treatment (4) got the higher isozyme expression that produced 6 bands. These results are in agreement with those of Castro and Pereira (2008) found higher enzymatic activity resulted from treating with thiamethoxam than control.

Determination of minerals content of coated cowpea seeds.

The results obtained in Table (6) revealed that the nitrogen, phosphor and potassium content of coated cowpea seeds (%) were significantly increased at treatment (5) and treatment (4) compared to untreated control after 12 months of storage period. These results are in harmony with the data Castro and Pereira (2008) who found that thiamethoxam had the capability of inducing physiological changes in plant. It can increase mineral nutrition of the plants, which promotes positive responses in plant development and productivity.

Determination of protein content, total free amino acid and total sugars.

The results obtained in Table (7) revealed that the total protein and Total sugars content of cowpea seedlings (%) were significantly increased at treatment (5) compared to untreated control after 12 months of storage period; but, total free amino acid was significantly reduced to interaction between the storage period (12 months) and seed coating treatments in treatment (5) compared untreated control seeds. These results are in harmony with Maity et al., (2015) who found that when cowpea seeds treated by fungicide, insecticide and nutrient mixture of N, P , K and other micronutrients, the coated seeds exhibited enhanced protein content and carbohydrate contents. Also Thobunluepop (2009) reported that when coated rice seeds by traditional fungicide and biological fungicide polymers performed the best of seed vigor increased sugar content.

Second experiment:

All treatments were done for soil fungi such as *Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani*. It is clear from the obtained results in Fig. (2) and Table (8) revealed that treatment (3), (4) and (5) were resistant to soil fungi. These results are in harmony with (Maienfisch et al., (2001) who reported that Thiamethoxam controls number of economically important crop pests with low use rates. Also (Falloon et al., 2000) reported that fungicides were developed for the protection of crops against diseases and pests. When fungicides are applied to the seeds, these chemicals had proven to be a form of crop insurance that protects investment of growers and this is performed by seed companies devoted to ensure that seed of high quality.

Table (4): Polyphenyl oxidase isozymes ideogram analysis of cowpea seedlings in the first seed storage period.

Rf	control	T1	T2	T3	T4	T5
0.041	++	+	++	++	++	++
0.123	+	+	+	++	++	++
0.297	+	-	+	+	+	+
0.416	-	-	-	-	+	+
0.521	-	+	-	-	-	-
0.557	-	+	-	-	-	-
0.626	++	+	++	++	++	++
0.685	++	-	++	++	++	++
0.743	++	-	++	++	++	++
0.834	+	-	-	+	+	+
0.919	+	-	+	+	+	+

-: absent, + : low density, ++ : moderate density and +++ : high density

Table (5): Polyphenyl oxidase isozymes ideogram analysis of cowpea seedlings in the third seed storage period.

Rf	control	T1	T2	T3	T4	T5
0.459	++	+	++	++	++	++
0.604	++	+	+	++	++	++
0.705	++	-	++	++	+++	++
0.825	+	-	+	+	+	+
0. 890	+	-	-	+	+	+
0.961	-	-	-	+	+	+

-: absent, + : low density, ++ : moderate density and +++ : high density

Table (6) Effect of seed coating treatments on Nitrogen, Phosphor and Potassium content (%) of cowpea seedlings.

Seed storage periods	Seed coating treatments	N (%).	P(%)	K(%)
4 months	Control	3.150 c	0.474 c	0.999 c
	T1	3.204 c	0.603 b	0.992 c
	T 2	3.412 b	0.598 b	1.033 b
	T 3	3.237 c	0.584 b	1.005 c
	T4	3.478 ab	0.710 a	1.082 a
	T5	3.635 a	0.720 a	1.065 a
12 months	Control	2.939 C	0.457 c	0.999 cd
	T1	3.043 bc	0.560 b	0.990 d
	T2	3.091 bc	0.565 b	1.012 bc
	T3	3.110 bc	0.569 b	0.993 d
	T4	3.218 b	0.683 a	1.035 a
	T5	3.412 a	0.705 a	1.029 ab

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

Table (7). Effect of seed coating treatments on total protein, total free amino acid and total sugars (%) of cowpea seedlings.

Seed storage periods	Seed coating treatments	Total protein content (%)	Total free amino acid content (%)	Total sugars content (%)
4 months	Control	19.68 d	0.067 a	12.47 c
	T1	20.02 d	0.068 a	12.71 bc
	T2	21.32 bc	0.057 c	12.49 c
	T3	20.32 cd	0.062 b	13.31 b
	T4	21.73 ab	0.053 d	13.43 b
	T5	22.72 a	0.061 b	14.65 a
12 months	Control	18.37 c	0.067 a	11.66 d
	T1	19.02 bc	0.069 a	12.06 c
	T2	19.32 bc	0.060 c	11.89 cd
	T3	19.43 bc	0.063 b	12.97 b
	T4	20.11 b	0.057 d	13.11 b
	T5	21.32 a	0.062 bc	13.74 a

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level.

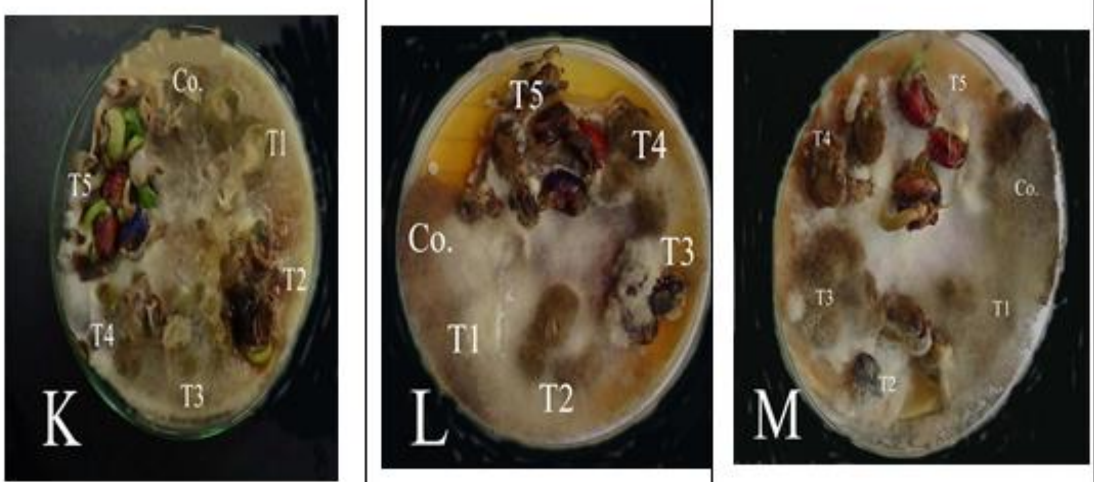

Fig. 2. Image of cultivar Teba seeds (dry seeds cowpea cv.) treated by polymers, fertilizers, insecticides and fungicides as seed coating, with Fungal soil Pathogen, treatment 5 (T5) was the most tolerable for infestation of Rhizoctonia Solani, Fusarium solani and Fusarium oxysporum, (K),(L) and (M), respectively.

Table (8) Effect of fungi soil in all seed coating treatments of cowpea seeds.

Treatments	*Fusarium oxysporum*	*Fusarium solani*	*Rhizoctonia solani*
Control	-	-	-
T1	-	-	-
T2	-	-	-
T3	+	+	+
T4	+	+	+
T5	++	++	++

- : susceptible, +: low tolerance, ++: moderately tolerance and +++: high tolerance

CONCLUSION

It could be concluded that the treatment (5) recorded the best values for the most studied characteristics. Seed production companies may be recommended to apply treatment (5): (Chitosan + 18-18-18 fertilizer + (thiamethoxam + mefenoxam+fludioxonil (CruiserMaxx)) + fludioxonil (Maxim)) to produce high-quality cowpea seeds and free from the insect injury. Also characterized by tolerance of soil fungal infection when planting.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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