

## **RESPONSE OF SOME MEDICAL PLANTS TO COMBINED INOCULATION WITH SOME N<sub>2</sub>-FIXING AND PHOSPHATE DISSOLVING MICROORGANISMS IN RELATION TO INSECT INFESTATION IN STORAGE**

**El-Zun, H. M.\* ; M. Nour El-Din\*\* and Sahar I. Ahmed\*\*\***

\* Dept. of Stored Product Pests Res., Plant Protection Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt.

\*\* Dept. of Microbiology, Soil, Water and Environmental Res. Institute, Giza, Egypt.

\*\*\* Dept. of Pesticides, Faculty of Agric., Kafr El-Sheikh University.

### **ABSTRACT**

Four medical plants pre-treated in the field with traditional, organic and biofertilizers as combined inoculation of some N<sub>2</sub>-fixing and phosphate dissolving microorganisms namely fenugreek, mustard, black cumin and nostortium, each plant material have four fertilization treatments were tested against *Sitophilus oryzae*. All sixteen plant materials were evaluated against the insect pest to study the degree of infestation on the tested materials of the 16 tested medical plant materials, most of them exhibited more tolerance than the other remained and had the lowest numbers of pests recorded monthly as Acarina and Insecta for nearly one year. These seeds mixed with wheat grains as protectants at two levels of 1 and 10% by weight. The results obtained showed that the degree of infestation and the mean of emerged adults of *S. oryzae* were affected by the plant species as well as the level and the kind of fertilizers. Inoculation of bio-organo fertilizer and / or chemical fertilizer changed some physical and chemical characters of tested plant species compared with control (traditional fertilization). Therefore, the differences for susceptibility of insect infestation under the different levels and the kind of fertilizer may be due to this cause. So, the type of fertilization may have an effect on the degree of insect infestation. Pirimiphos-methyl included in the present study as a recommended chemical insecticide against stored product insects for comparison the chemical insecticide tested exhibited the highest effect on the all studied parameters.

Thus we recommend mixing wheat grains with fenugreek by the ratio of 90 : 10 to result on decrease of insect infestation without addition of chemical pesticides. Fenugreek addition to wheat grains may also improve nutritional value of the resulted flour, to shure these findings further experiments are needed.

### **INTRODUCTION**

Use of chemical fertilizers at extremely high rates for long periods in agricultural processes reduced potential activity of microflora and the stability of soil organic matter (Pokorna, 1984). They also increase residual toxic components in the soil and plants, like biuret from urea fertilizer, and generate serious complications on the environment. In recent years, the use of synthetic pesticides in crop protection resulted in potential hazards for mammals, disturbances of the environment, pest resistance to pesticides and lethal effects on non-target organisms, agroecosystems in addition to direct toxicity to users (Prakash and Rao, 1986 and 1987).The uncontrolled use of

these synthetic insecticides also causes great hazards for environment and consumers due to residual property (EL-Kamali,2009),Therefore,it is an urgent need to develop insecticides which should be ecologically,biodegradable and cause no toxicity in non-target animals i;e humans. In this regard,many plants products have been evaluated for their insecticidal properties against different stored-grain pests(Mondal and Khalequzzaman ,2010 ). In the present time, the attention has been paid towards use of microorganisms as fertilizers for medicinal plants to get healthy products. Many of microorganisms act together in mutual manner encouraging their survival and activity (Alexander, 1977). Also, it has become necessary to search for alternative means of pest control with lower mammalian toxicity which can minimize the use of these synthetic chemicals. Many insects are unable to infest certain plants because of the presence of particular noxious substances (Fraenkel, 1969). Plants may be considered as a biosynthetic laboratory in which many metabolic processes take place, not only for chemical compounds (carbohydrate, protein and fats) but also for a multitude of secondary compounds (glycosides, alkaloids, and volatile oils) that exert a physiological effect. These chemical compounds give plant drugs with therapeutic properties. Many scientific workers investigated the utilization of plant materials (whole and their derivatives) as crop protectants before and post-harvest particularly stored products where store conditions are easily controlled (Ahmed and Koppel, 1986, Afifi *et al.*, 1988 & 1989, Islam *et al.*, 1989, El-Aidy and Helal, 1997). Many authors found that chemical composition responded to the different levels and the kind of chemical and biofertilizers (Balba, 1968, Lavoy and Hageman, 1970, Youssef and Salem, 1976). The relationship between insect infestation and chemical composition of grains was investigated by many authors, (Caswell, 1961, Warchalewski *et al.*, 1993 and Zein and Abo Arab, 2000). Hence, the medicinal plants are considered the main source of pharmacological, and therapeutically active agents. Therefore, the objectives of this study were:

1. To survey some organisms attack tested medicinal plants.
2. To select the promising medicinal plant materials which many act as stored product protectants.
3. To study the biology of an important insect of wheat grains, *S. oryza* exposed to a mixture of wheat grains with and medical seeds at levels of 1 and 10% wt/wt of grains.
4. To evaluate the effect of inoculation of some medicinal seeds with a mixture of microorganisms specially on chemical composition compared to that produced by traditional chemical and organic biofertilization and the influence of this on raising resistance of produced seeds against insect infestation throughout storage period.

## **MATERIALS AND METHODS**

The present work was carried out in the laboratory of Stored Product Research Department, Sakha Research Station Kafr El-Sheikh.

Four medical plant materials pre-treated in field with chemical and biofertilizers namely: *Trigonella foenum-greacum*, (Fenugreek); *Nigella sativa*

L. (black cumin); *Lepidium sativum* L. (nostortium), and *Brassica alba*, (mustard).

**Seeds:** seeds of the studied plant species were kindly provided by Horticulture Research Institute, Sakha Agricultural Research Station.

**Inoculum:** composed of three types of microorganisms: *Azotobacter sp.*, *Bacillus megatherium* and *Rhizobia* were kindly obtained from bacteriology lab. Giza and Sakha Agricultural Research Station, and maintained on the proper agar medium. Potassium was added as 50 kg K<sub>2</sub>O/fed. to all experiments as potassium sulphate. Completely randomized design with four replicates was used for each plant species. Inoculation process was conducted by mixing these microbial types with the seeds of the studied plants before sowing at the rate of 200 g peat-based inoculum/feddan.

**The treatments were assigned as following:-**

1. Traditional, control was formed of 100% chemical fertilizer (100 kg N/Fed. as urea) and 30 kg P<sub>2</sub>O<sub>5</sub>/fed. as calcium super phosphate 15.5%.
2. **Organic:** obtained 10 ton/fed., poultry manure + 100% chemical fertilizers, with rates of traditional control.
3. **Bio-1:** 10 tons poultry manure + inoculation with mixed inoculum and obtained 25 kg/fed N and 15 kg/fed. P<sub>2</sub>O<sub>5</sub> for non-leguminous crops and 12.5 kg/fed., N and 15 kg/fed. P<sub>2</sub>O<sub>5</sub> for leguminous crops.
4. **Bio-2:** 10 tons poultry manure + inoculation with mixed inoculum and obtained 50 kg/fed N and 15 kg/fed. P<sub>2</sub>O<sub>5</sub>/fed., for non-leguminous crops and 25 kg/fed., N and 15 kg/fed. P<sub>2</sub>O<sub>5</sub>/fed., for leguminous ones.

Poultry manure at the rate of 10 ton/fed. was added to all treatments (=12) except for traditional (control = 4).

At harvest time, all plant species (16 treatments) were harvested for chemical determination. Randomized samples of seeds from each treatment were taken, cleaned by excluding foreign materials and dried, then ground to a fine powder and kept for chemical analysis.

**Insecticide:**

**Pirimiphos-methyl.(Actellic) 50%(EC).**

**Chemical name:**O-2 diethyl amino-6-methyl-pyrimidin-4-yl-o,o-dimethyl phosphorothioate. was used as a reference in this study. The concentration of LC<sub>90</sub> (0.0052 mg/kg) (which kills 90% of the tested insects) was used mixing with media, % Mortality, % damage and progeny were estimated and recorded.

**Chemical analysis of seeds:** Ten grams of each of the powdered seeds were successively exhausted with petroleum ether at 40-60 °C and ethanol 10% respectively according to the Egyptian pharmacopeia procedures (1953). Both extracts were subjected to phytochemical screening for their chemical constituents according to the procedures predescribed by Balbaa *et al.*, (1976).

**Determination of the essential constituents:** The essential constituents of the alcoholic extracts for each drug were determined. The total alkaloids content for fenugreek and black cumin were determined according to Koutb (1996) and Abdel-Hady (1998), while the thioglycoids content of nostortium

and mustard were determined in the same extract according to the Egyptian Pharmacopeia (1953) procedure.

**Survey studies:**

One half kilogram of every plant material previously mentioned were first tested and cleaned up thermally to be sure that it is free from any insect stages and were kept in a glass jars covered with cheese cloth and closed with rubber band, and kept at room temperature for approximately one year. The monthly mean temperature ranged between 13 to 20 °C in winter months while it ranged from 23 to 30 °C in summer time.

Monthly samples were taken from every container. At every sampling date, 3 replicates represented each sample of the 16 tested materials. Every chosen sample of 40 g were taken, put in a cheese cloth of 20 x 20 cm closed from its upper edge by a piece of thread and transferred directly to a battery of modified Tullegram Funnel adapted with a 40 watt bulbs, the separated animals and insects were collected in a petri-dish of 10 cm in diameter containing water and the results recorded directly. The process of extraction took place for 48 hours. It was also planned to mark every Tullegram Battery to hold the same materials all over experimental time. Before and after mounting samples, funnels and sieves had to be thoroughly cleaned up by using a suitable brush and cleaning cloth. The extracted insects and mites were transferred to small vials and thoroughly closed. Ethyl alcohol 70%, and some drops of glycerol were added to every vial. All important data had to be recorded on every vials and the bulk of vials were kept in jars containing ethyl alcohol thoroughly closed. A collection of either jars containing resultant pests and cleared slides were kept for identification and added to the Stored Product Pests Department collection. The seed species which had the least number of pests were selected to complete further studies.

The wheat cultivar (Sakha 8) was used for rearing the rice weevil, *S. oryzae* L. under laboratory conditions of 25 ± 5 °C and 65 ± 5 % R.H.

**Susceptibility to infestation:-**

For each trial, 20 insects (1-2 weeks old) were placed into a 250 glass jars containing 20 g of each tested seeds (16 x 3 replicates for each). A cheese cloth was used to cover the Jars tops. The percent of mortality was recorded and corrected with Abbott's formula, (1925). The live insects were removed after 20 days of infestation. Emerging offspring s, were observed seven weeks after initial infestation and emerging adults were removed on alternate days until emergence was completed within 60 days (Ivbijaro *et al.*, 1985). The number of emerging adults was used as an indicator for the susceptibility of tested medicinal materials to infestation with *S. oryzae*. As a natural preferred media, wheat grains were used as a control.

**Progeny study:**

The promising medicinal materials as stored product protectants (that produced high mortality and little offspring) were selected to carry out further studies on progeny of *S. oryzae*. Batches of 20 g of wheat grains were mixed with two ratios (1% and 10%) for all selected materials separately (sound seeds). The mixtures were placed into a glass jars (1/4 kg). The jars were kept under laboratory conditions of 25 ± 5 °C and 65 ± 5 % R.H. Unsexed ten

adults of rice weevil of (0-1 week old) were taken from a laboratory culture and introduced into each jar. Three replicates of each ratio were tested. Jars were covered with muslin cloth. Jars free from selected materials were used as a control. Twenty days post-treatment, all adult weevils were removed and mortality count was recorded. Emerging adults were recorded till 60 days after application and damage to seeds was assessed by the number of exit holes observed in each 20 g of grains.

All data were subjected to the statistical analysis as usual technique of analysis of variance (ANOVA) mentioned by Sendecor and Cochran (1967).

## RESULTS AND DISCUSSION

### Phytochemical screening of the extracts:

All the tested plants showed high positive results with sterols and /or triterpines as well as carbohydrates with all treatments (Table 1).

**Table (1): Phytochemical screening for extracts of the different medicinal plant seeds (cited from Nour El-Din *et al.*, 2005)**

| Treatments            | Alkaloids | Carbohydrates | Glycosides |         |      |             | Sterols and/or triterpines | Volatile oil |
|-----------------------|-----------|---------------|------------|---------|------|-------------|----------------------------|--------------|
|                       |           |               | Flavonoid  | Saponin | Thio | Cyanophoric |                            |              |
| <b>Fenugreek</b>      |           |               |            |         |      |             |                            |              |
| Traditional (control) | +         | ++            | +          | +       | -    | -           | ++                         | ±            |
| Organic manure        | ++        | ++            | ++         | ++      | -    | -           | ++                         | +            |
| Bio-1                 | +++       | ++            | ++         | ++      | -    | -           | ++                         | +            |
| Bio-2                 | ++        | ++            | ++         | ++      | -    | -           | ++                         | ±            |
| <b>Black cumin</b>    |           |               |            |         |      |             |                            |              |
| Traditional (control) | ±         | ++            | +          | +       | -    | -           | ++                         | +            |
| Organic manure        | +         | ++            | +          | +       | -    | -           | ++                         | +            |
| Bio-1                 | +         | ++            | +          | +       | -    | -           | ++                         | ++           |
| Bio-2                 | +         | ++            | +          | +       | -    | -           | ++                         | +            |
| <b>Nostortium</b>     |           |               |            |         |      |             |                            |              |
| Traditional (control) | ±         | ++            | -          | -       | +    | ±           | ++                         | -            |
| Organic manure        | ±         | ++            | -          | -       | ++   | +           | ++                         | -            |
| Bio-1                 | +         | ++            | -          | -       | ++   | +           | ++                         | -            |
| Bio-2                 | ±         | ++            | -          | -       | +    | ±           | ++                         | -            |
| <b>Mustard</b>        |           |               |            |         |      |             |                            |              |
| Traditional (control) | -         | ++            | -          | -       | +    | -           | ++                         | +            |
| Organic manure        | -         | ++            | -          | -       | ++   | -           | ++                         | +            |
| Bio-1                 | -         | ++            | -          | -       | ++   | -           | ++                         | ++           |
| Bio-2                 | -         | ++            | -          | -       | ++   | -           | ++                         | +            |

(-): absent, (±): present in traces, 1-10%. (+): present in ranges of 11 - 30 %.

(++): present in ranges of 31-50 %.

(+++): present in very high concentration, 51-70 %.

Traditional : 100% N and P

Organic manure : 10 tons Poultry manure + 100% N and P.

Bio-1 : 10 tons Poultry manure + microbial inoculation + 25% N and P.

Bio-2 : 10 tons Poultry manure + microbial inoculation + 50% N and P.

Alkaloids were detected in fenugreek, black cumin and nostortium. They were present in high concentrations in fenugreek but it in traces in case of neigella. Flavonoids were highly found in fenugreek, but low in black cumin. Saponin glycoside was present only in fenugreek and black cumin, also fenugreek was superior. Thioglycosides, were remarked in both nostortium and mustard while cyanphoric glycoside was detected only in nostortium. Fenugreek, black cumin and mustard showed volatile oils while nostortium was free from them, in general organic fertilization treatments showed high concentrations over traditional chemical fertilization treatment, especially Bio-1 treatment.

**Percent of the essential constituents:-**

The calculated constituents in case of fenugreek and black cumin; were alkaloids while thioglycosides were calculated in case of nostortium and mustard (Table 2).

As a common conclusion Bio-1 and Bio-2 showed higher results. All the bio treatments gave higher records than the traditional.

Many studies were in accordance with the present results. However Sharma *et al.*, (1997) found that the increase, of nitrogen fertilizer application reduced seed oil content of *Brassica* sp. Chauhan *et al.*, (1996) recorded an increase in oil content of Indian mustard with *Azotobacter* and *Asospirillum* inoculation. Also, Abdelgany *et al.*, (1999) found that inoculation with *rhizobia* increase fat content of fenugreek seeds. Other authors, like El-Sawy *et al.*, (1998) reported similar effect on other chemical constituents. They found that inoculation of *Amini visnaga* with *Azotobacter*, *Azospirillum*, *Bradyrhizobium* and Mahershawari *et al.*, (1995) on *Hyoscyamus niger* indicated that the inoculation with *Azotobacter chroococcum* + 40 kg N/ha, gave the highest alkaloid yields. On the other hand, inoculation with *Azotobacter* + *Azospirillum* and organic amendements increased hyosyamine content of *Hyoscymus muticus* L. El-Sawy *et al.*, (1986) and Harray and Amara (1998) found that biosynthesis of anthocyanin is superior in case of inoculation of *Hibiscus sabdariffa* with *rhizobia* than inoculation with *Azotobacter* and *Azospirillum*.

Hendawy (2008) determined the effect of three levels of compost tea (100, 200 and 300 ml/L compost) and two levels of mineral fertilizer (100, 100, 50 and 200, 200, 50 NPK) or their combinations on chemical constituents of *Plantage arenaria*. The various fertilizers levels caused significant promotion for mucilage content as well as P, K, F, Cu, Zn and Mn. Highest level of organic and mineral fertilizers increased significantly total carbohydrates content.

Many research workers studied the effectiveness of some plant components derived from plant origin against some of stored product insects.

Hosozawa *et al.*, (1974) (diterpenes), Su (1977) (alkaloids), Su and Robert, 1981) (amids), Abbassy, 1982 (alkaloids), Koul, 1983 (limonoids), Gurguis *et al.*, 1989 (alkaloids), Zidan *et al.*, 1994 (alphapinene, limonine, B-pinene and (ineal), Abd El- All *et al.*, 1999 (alkaloids), and Mohmed, 2001 (sterol, triterpenes, alkaloids, flavonoids, and glycosides), they confirmed that these components have toxic, antifeeding and other bioactivities against the tested insects.

**Table (2): Biofertilizer effect on the percentages of essential constituents of different medicinal plant seeds**

| Treatments     | Fenugreek* | Black cumin* | Nostortitim** | Mustard** |
|----------------|------------|--------------|---------------|-----------|
| Traditional    | 0.12       | 0.03         | 0.35          | 0.22      |
| Organic manure | 0.17       | 0.06         | 0.44          | 0.24      |
| Bio-2          | 0.20       | 0.07         | 0.59          | 0.33      |
| Bio-1          | 0.18       | 0.06         | 0.48          | 0.30      |

\* Alkaloids

\*\* Thioglycosides

Traditional : 100% N and P

Organic manure : 10 tons Poultry manure + 100% N and P.

Bio-1 : 10 tons Poultry manure + microbial inoculation + 25% N and P.

Bio-2 : 10 tons Poultry manure + microbial inoculation + 50% N and P.

Results in Tables (1 and 2) showed that the tested seeds contained some chemical components which have killing, antifeeding, repellent and attractive properties such as alkaloids, terpenoids, flavinoids, saponines and triterpenes. The tested seeds had these components at different degrees. The biological fertilization treatments in the present study changed the levels of some chemical components Tables (1 and 2) compared to chemical fertilization.

**Pest survey and tested materials:**

Data obtained in Table (3) showed that the four tested medicinal seeds were exposed to different groups of pests. *Sitophilus oryzae* and *Stegobium paniceum* were recorded on fenugreek seeds while black cumin have *Trogium pulsatorium*. Nostortium have no numbers either insects or mites at the all fertilization treatments.

**Table (3): Numbers of all insects and mites on medicinal plant seeds pretreated with bio, organo and mineral fertilization**

| Seed species | Fertilization treatments | Infestation                |    |                                                                 |    |
|--------------|--------------------------|----------------------------|----|-----------------------------------------------------------------|----|
|              |                          | Insects                    | N. | Mites                                                           | N. |
| Fenugreek    | Traditional (control)    | -                          |    | -                                                               |    |
|              | Organic manure           | <i>Sitophilus oryzae</i>   | 1  | -                                                               |    |
|              | Bio-1                    | <i>Stegobium paniceum</i>  | 1  | -                                                               |    |
|              | Bio-2                    | -                          |    | -                                                               |    |
| Black cumin  | Traditional (control)    | <i>Trogium pulsatorium</i> | 3  | -                                                               |    |
|              | Organic manure           | <i>Trogium pulsatorium</i> | 1  | -                                                               |    |
|              | Bio-1                    | -                          |    | -                                                               |    |
|              | Bio-2                    | -                          |    | -                                                               |    |
| Nostortium   | Traditional (control)    | -                          |    | -                                                               |    |
|              | Organic manure           | -                          |    | -                                                               |    |
|              | Bio-1                    | -                          |    | -                                                               |    |
|              | Bio-2                    | -                          |    | -                                                               |    |
| Mustard      | Traditional (control)    | <i>T. pulsatorium</i>      | 5  | <i>Cheyletus malaccensis</i><br><i>Tyrophagus putrescentiae</i> | 5  |
|              | Organic manure           | <i>T. pulsatorium</i>      | 3  | <i>C. malaccensis</i>                                           | 3  |
|              | Bio-1                    | <i>T. pulsatorium</i>      | 3  | <i>I. putrescentiae</i>                                         | 2  |
|              | Bio-2                    | <i>T. pulsatorium</i>      | 5  | <i>C. nalaccensis</i>                                           | 1  |

(-) = No pest infestation

N = number.

Traditional : 100% N and P

Organic manure : 10 tons Poultry manure + 100% N and P.

Bio-1 : 10 tons Poultry manure + microbial inoculation + 25% N and P.

Bio-2 : 10 tons Poultry manure + microbial inoculation + 50% N and P.

No number of mites species was recorded on the three mentioned medicinal seeds which were pretreated with bio-organo-chemical fertilizers, before sowing. It is also noted that bio-treatment especially Bio-1 remarkably decreased number of infestation in all studied plant species.

**Table (4): A list of the insects and mites surveyed from medicinal plant seeds**

| Scientific name                           | Family         |
|-------------------------------------------|----------------|
| <b>Acarina</b>                            |                |
| <i>Cheyletus malaccensis</i> (Oudemons)   | Cheyletidae    |
| <i>Tyrophagus putrescentiae</i> (Schrank) | Acaridae       |
| <b>Insecta</b>                            |                |
| <i>Trogium pulsatorium</i> (L.)           | Liposcelidae   |
| <i>Stegobium pulsatorium</i> (L.)         | Anobiidae      |
| <i>Sitophilus oryzae</i> (L.)             | Cureculionidae |

**Table (5): % Mortality and emergence of *Sitophilus oryzae* adults exposed to different medicinal plant seeds pretreated with bio, organo, and chemical fertilization 5 days posttreatment**

| Seed species          | Fertilization treatments | % Mortality | Adults emergence | % reduction |
|-----------------------|--------------------------|-------------|------------------|-------------|
| Fenugreek             | Traditional (control)    | 80          | 0                | 100         |
|                       | Organic manure           | 75          | 0                | "           |
|                       | Bio-1                    | 70          | 0                | "           |
|                       | Bio-2                    | 65          | 0                | "           |
| Black cumin           | Traditional (control)    | 90          | 0                | "           |
|                       | Organic manure           | 85          | 0                | "           |
|                       | Bio-1                    | 100         | 0                | "           |
|                       | Bio-2                    | 100         | 0                | "           |
| Nostortium            | Traditional (control)    | 35          | 0                | "           |
|                       | Organic manure           | 87          | 0                | "           |
|                       | Bio-1                    | 100         | 0                | "           |
|                       | Bio-2                    | 100         | 0                | "           |
| Mustard               | Traditional (control)    | 95          | 0                | "           |
|                       | Organic manure           | 95          | 0                | "           |
|                       | Bio-1                    | 90          | 0                | "           |
|                       | Bio-2                    | 90          | 0                | "           |
| Wheat grain (control) |                          | 0           | 13.7             | -           |

(-) = No pest infestation.

Traditional : 100% N and P

Organic manure : 10 tons Poultry manure + 100% N and P.

Bio-1 : 10 tons Poultry manure + microbial inoculation + 25% N and P.

Bio-2 : 10 tons Poultry manure + microbial inoculation + 50% N and P.

Results obtained in Table (6) demonstrated that all levels of treatments, medicinal seeds increased the mortality, reduction of progeny and consequently decreased the damage of wheat grains compared to the control (wheat grains only).% Mortality increased when the exposure period increased.



**Table (6): Effect of mixing of medicinal plant seeds and wheat grains (at levels 1 and 10%) of Mortality, % emerged adults and % damage of *Sitophilus oryzae* adults**

| Seed species      | Fertilization treatment | % Mortality |     |         |     | Mean adults emerged |        |        |        | % damage |        |
|-------------------|-------------------------|-------------|-----|---------|-----|---------------------|--------|--------|--------|----------|--------|
|                   |                         | 5 days      |     | 10 days |     | At 1%               | % R    | At 10% | % R    | 1%       | 10%    |
|                   |                         | 1%          | 10% | 1%      | 10% |                     |        |        |        |          |        |
| Fenugreek         | Traditional (control)   | 10          | 75  | 25      | 75  | 13                  | 41.7d  | 3      | 86.55c | 10.3b    | 3.6bc  |
|                   | Organic manure          | *           | *   | *       | *   | *                   | *      | *      | *      | *        | *      |
|                   | Bio-1                   | 16          | 80  | 27      | 86  | 9                   | 59.64c | 2      | 91.03b | 8.2b     | 3.2bc  |
|                   | Bio-2                   | 19          | 84  | 29      | 89  | 8                   | 64.13c | 2      | 91.03b | 7.0b     | 2.1c   |
| Black cumin       | Traditional (control)   | *           | *   | *       | *   | *                   | *      | *      | *      | *        | *      |
|                   | Organic manure          | 15          | 20  | 25      | 30  | 13.5                | 39.46d | 7.5    | 66.37d | 9.16b    | 6.94b  |
|                   | Bio-1                   | 21          | 59  | 32      | 81  | 10                  | 55.16c | 3      | 86.55c | 7.6b     | 4.9b   |
|                   | Bio-2                   | 25          | 61  | 37      | 80  | 10                  | 55.16c | 3      | 86.55c | 7.0b     | 3.1bc  |
| Nostortium        | Traditional (control)   | *           | *   | *       | *   | *                   | *      | *      | *      | *        | *      |
|                   | Organic manure          | 15          | 25  | 20      | 35  | 4.5                 | 79.82b | 2.0    | 91.03b | 5.27c    | 3.05bc |
|                   | Bio-1                   | 25          | 25  | 30      | 45  | 2.5                 | 88.79b | 2.0    | 91.03b | 4.72c    | 4.16b  |
|                   | Bio-2                   | 10          | 20  | 20      | 35  | 12.5                | 43.95b | 4.0    | 82.06c | 7.22b    | 4.16b  |
| Mustard           | Traditional (control)   | *           | *   | *       | *   | *                   | *      | *      | *      | *        | *      |
|                   | Organic manure          | *           | *   | *       | *   | *                   | *      | *      | *      | *        | *      |
|                   | Bio-1                   | 40          | 50  | 50      | 60  | 4.5                 | 79.82  | 1      | 95.52b | 5.27c    | 3.61bc |
|                   | Bio-2                   | 43          | 52  | 57      | 66  | 4.0                 | 82.06  | 1      | 95.52b | 3.8c     | 3.1bc  |
| Pirimiphos-methyl |                         | 100         | 100 | 100     | 100 | 0                   | 100a   | 0      | 100a   | 0c       | 0c     |
| Wheat grains only |                         | 0           | 0   | 0       | 0   | *                   | 22.3   | *      | *      | 18 a     | *      |

In each column, means followed by a common letter are not significantly different at the 5% level according to DMRT.

\* = not tested

Traditional : 100% N and P

Organic manure : 10 tons Poultry manure + 100% N and P.

Bio-1 : 10 tons Poultry manure + microbial inoculation + 25% N and P.

Bio-2 : 10 tons Poultry manure + microbial inoculation + 50% N and P.

Generally, all studied parameters (%mortality, % damage, and total number of emerged adults) differed among each other, and that may be due to some physio-chemical characters of the tested seed species and also to the fertilization treatments which changed the chemical composition of the tested seeds.

Accordingly, the tested parameters (the percentages of mortality, damage and reduction) affected by these treatment, it is worthily to conclude that the tested medicinal seeds which arised from seeds pre-treated with biological fertilizers before sowing significantly decreased total adult emergence damage percentage and increased mortality percentage especially with fenugreek and black cumin followed by mustard and nostortium with bioorgano fertilizer treatment at 10 days posttreatment. The

chemical insecticide, pirimiphos- methyl had the highest effect on the all tested parameters comparing with the tested medicinal seeds, where it caused 100 % mortality, 100% reduction in progeny and zero damage when admixed with wheat grains at level of LC<sub>90</sub>.

Consequently, the medicinal seeds tested can play an important role for protecting the stored products as one of control means. This might minimize the cost of pest control and hazards on human and environment as a result of pesticides application. The present results are in agreement with Ahmed and Koppel (1986) who found that 80% of cardamom growers and 25- 30% of vegetable growers used neem seeds for nematode control. Neem leaves were used in the storage of wheat, rice, sorghum and millet.

Tanzubil (1987) evaluated the effectiveness of neem fruit dust at 2, 5 and 10%, neem leaf dust at 5 and 10% for the protection of stored cowpeas (*Vigna unguiculata*) from *Callosobruchus maculatus*.

Jilani and Su (1983) studied the effectiveness of parts of three plants, the rhizomes of turmeric and the leaves of neem and fenugreek as repellent agent to adults of *T. castaneum* (Hbst.), *S. granarius* (L.) and *Rhyzopertha dominica* (F.).

Su (1984) used ground black pepper to surface treat wheat which was then infested with *S. oryzae* (L.).

Mousa *et al.*, (2011) tested chemical composition of extracts from black pepper, *Piper nigrum* L. and physic nut, *Jatropha curcas* L against *Sitophilus oryzae* L under laboratory conditions They showed that *P. nigrum* and *J. curcas* extracts were able to protect stored grain.

#### **Acknowledgement**

Thanks are due to Dr. Abo Arab, R.B. Dep. of Stored Product Pests Research Institute, Agric. Res. Cent. Dokki, Giza, Egypt for providing facilities during the preparation of this work.

#### **REFERENCES**

- Abbasy, M.A. (1982). Naturally occurring chemicals for pest control. III. Insecticidal and synergistic alkaloid isolated from *Schinus terebinthifolius* Raddi. Med. Fac. Landbou WW. Rijksuniv. Gent.4712-617-626 .
- Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. J. Econ. entomol. 18: 265-267.
- Abd El-All, S.M.; H.A. Zedan and S.Y. Abd El-Naby (1999). Toxicity and biochemical response of house sparrows, *Passar domesticus niloticus* to some plant extracts and lebacide pesticide. The 2<sup>nd</sup> Int. Conf: of Pest Control, Mansoura, Egypt, Sept., 1999.
- Abdelgany M.E., E.A.E. El-Sheikh and N.O. Mukhtar (1999). The effect of *rhizobium* inoculation and chemical fertilization on seed quality of fenugreek. Food Chemistry, 64:289-293.

- Abdel-Hady, N.M. (1998). Pharmacognostical Investigation and Biological Verification of Some Recipeo and Preparations. M.D. Thesis in Pharmaceuical Sci. "Pharmacognosy".
- Afifi, F.A.; Hekal, A.M. and Salem, M. (1988). Fenugreek seed extracts as protect ants of wheat grains against certain stored product insects. *Annals of Agricultural Science, Cairo.* 33(2): 1331-1342.
- Afifi, F.A.; Salem, M. and Hekal, A.M. (1989). Insecticidal properties of the extracts of lupine seed and caraway fruits against some stored product insects. *Annals of Agricultural Science, Cairo.* 34(1): 401-414.
- Ahmed, S. and Koppel, B. (1986). Use of neem and other botanical materials for pest control by farmers in India: summary of findings. Eschborn. German Federal Republic; Deutsche Gese 11 schaft fur Technische Zusammenarbeit. 623-626.
- Alexander, M. (1977). Interaction among species, In: Introduction to Soil Microbiology . John Wiiey and Sons Inc. P: 405 - 409.
- Balba, A.M. (1968). Soil fertility. Dar El-Kotob El-Gameia Alex. Egypt.
- Balbaa S.I., S.H. Helal and A.Y, Zaki (1976). Medicinal plant constituents" 2<sup>nd</sup> Ed. General Organization of University and School Book.
- Caswell, G.H. (1961). The infestation of cowpeas in the Weatern Region of Nigeria. *Trop. Sci.*, 3(4): 154-158.
- Chauhan, D.R., P. Shashi; R. Mangat; S. Paroda and M. Ram (1996). Response of Indian mustard (*Brassica juncea*) to biofertilizers, sulphur and nitrogen fertilization. *Indian J. of Agron.*, 41 : 620-623.
- El-Aidy, Nadia, A. and R.M.Y. Helal (1997). Efficiency of four natural materials as protectants for stored wheat grains against *S. oryzae* (L) and their effects on quality of grains. *J. Agric. Sci. Mansoura Univ.*, 22(12): 4217-4227.
- El-Kamali,H.H.,(2009).Effect of certain medicinal plants extracts against storage pest, *Tribolium castaneum* Herbst.Eur.J.sustain Agric.,3:139-142.
- El-Sawy, M., E.A. Saleh; A.M. El-Boroffosy; T.H. Nokhaf; I. Fendrik and M.S. Sharaf (1998). Effectiveness of dual inoculation with diazotrophs and vesicular-arbuscular mycorrhizae on the growth and khellin content of *Ammi visnaga*. *Arab-Univ. J. Agric. Sci.*, 6: 357-371.
- El-Sawy, M., E.A. Saleh; M.K. Abdel Fattah; M.A. Borollosy and M.S. Sharaf (1986). Hyoscyamine content of *Hyoscyamun muticus* L. as affected by inoculation with *Azotobacter* and/or *Azospirillum* strains. Proc 2<sup>nd</sup> Conf, AABNF, Cairo, Egypt. 498-513.
- Fraenkel, G. (1969). Evaluation our thoughts on secondary plant substances. *Entomologia Exp. Appl.* 12: 473-486.
- Gurguis, M.W.; K.M. Gouhar; W.M. Watson; N.A. Ibrahim and R.M. Salem (1989). Separation and identification of biological active components of two wild plants against *Myzus persicae*, *S. littoralis*. Pros. 1<sup>st</sup> Int. Conf. Econ. Ent, 11: 477.
- Harridy, I.M.A. and A. Amara (1998). Effect of presowing inoculation of seeds by nitrogen fixed bacteria on growth fruit production, sepals yield and the chemical composition of roselle plants. *Egypt J. Appl. Sci.* 13(6): 217-231.

- Hendawy, S. F. (2008). Comparative study of organic and Mineral fertilization on *Plantago arenaria* plant. J. Applied Science Research, 4(5): 500-506.
- Hoszawa, S., N. Koto, K. Munakata and Y. L. Ghen (1974). Antifeeding active substances for insect in plant. Agr. Biol. Chem. 38(5): 1045-1048.
- Islam, N.; Bhuiyah, M. I. M.; Begum, A. and Karim, M. A. (1989). Comparative efficacy of different material against *Sitophilus oryzae* L. infesting maize seeds in storage. Bangladesh Journal of Zoology. 17(2): 175-178.
- Ivbijaro, M. F.; C. Ligan and A. Youdeowel (1985). Control of rice weevil, *Sitophilus oryzae* (L.) in stored maize with vegetable oil. Agric., Ecosys. And Environ., 14; 237-242.
- Jilani, G. and H. C. F. Su (1983). Laboratory studies on several plant materials as insect repellants for protection of cereal grains. Journal of Economic Entomology. 76(1): 154-157.
- Koul, O. (1983). Feeding deterrence induced by plant liminoids in the larvae of *Spodoptera littoralis* (F.). Z. Fur Angew. Entomol. 95(8): 166-171.
- Koutb, S. K. I. (1996). Isolation and characterization of natural principles having anti-inflammatory activity. Ph.D. Thesis in pharrn. Sci. (Pharmalognos).
- Lavoy, I. D. and H. R. Hageman (1970). Relationship of nitrate reduction activity to grain protein product in wheat. Crop Sci. 10: 280-285.
- Mahereshwari, S. K.; S. K. Gangarde and R. K. Sharma (1995). Differential responses of *Azotobacter* and nitrogen on bio-mass and oil yield of palmarosa. Crop Res., 10 (3): 356-359.
- Mohamad, A. A. S. (2001). Evaluation of some plant extracts as protectants Against some of stored product pests. M.Sc. Fac. of Science (Girls). Al-Azhar University. Cairo, Egypt.
- Mondal, M. and M. Khalequzzaman, (2010). Toxicity of naturally occurring compounds of plant essential oil against *Tribolium castaneum* (Herbst). J. Biol. Sci., 10: 10-17.
- Mousa, K., R. M. Awang<sup>1\*</sup>, D. Omar<sup>1</sup>, M. Rahmani<sup>2</sup> and S. Rezazadeh. (2011) Tropical medicinal plant extracts against rice weevil, *Sitophilus oryzae* L. J. of Medicinal Plants Res. Vol. 5(2), pp. 259–265.
- Nour El-Dein, M. Younis, S. I. and Souzan, M. Moustafa (2005). Response of some medicinal plants to inoculation with N<sub>2</sub> fixing and phosphate Dissolving Microorganisms. Minufiya J. Agric. Res. 30(1): 297-315.
- Pokorna, K. J. (1984). Effect of long term fertilization on the dynamics of changes of soil organic matter. Zentrblatt Fur Mikrobiologie. 139 (7): 497-504 (Soil and Pert. Abst. 45 : 3225).
- Prakash, A. and J. Rao (1986). Evaluation of plant products as antifeedants against the rice storage insects. Proc. Symp. Resid and Environ. Pollution: 201-205.
- Prakash, A. and J. Rao (1987). Use of chemicals as grain protectants in storage ecosystem and its consequences. Bull. Grain. Tech. 25(1): 65-69.
- Sendecor, G. W. and W. G. Cochran (1967). Statistical methods. 6<sup>th</sup> ed. Iowa St Uni. Press. U.S.A.

- Sharma, S.K.; Rao, DSRM and S.K. Gupta (1997). Effect of crop geometry and nitrogen on quality and oil yield of *Brassica* species. Indian J. Agron., 42: 498-501.
- Su, H.C.F. (1977). Insecticidal properties of black pepper to rice weevils and cowpea weevils. J. Econ. Entomol. 70(1): 18-21.
- SU, H.C.F. (1984). Evaluation of black pepper as a protect ant of wheat in storage against rice weevils *Sitophilus oryzae*. J. Ga. Entomol Soc. 19(1): 45-48.
- SU, H.C.F. and H. Robert (1981). Isolation, identification and insecticidal properties of *Piper nigrum* amides, J. Agric. Food Chem.29(1):115
- Tanzubil, P.B. (1987). The use of neem products in controlling the cowpea weevil, *Callosobruchus maculatus*. Nyankpala Agric. Exp. Sta., Crops Res. Inst., 517-523.
- Warchalewski, R.; Piasecka-Kwiatkowska, D.; Nawrot, J. and Winiiecki, Z. (1993). Natural protection system of cereal grain against stored pests-myth or fact. Ochrona Roslin. 37(10): 11-12.
- Youssef, S. and A. Salem (1976). Response of wheat to several sources and rates of zinc. Alex. Journal Agricultural Research. 42(1): 47-53.
- Zein, F.I. and Abo-Arab, R.B. (2000). Combined effect of bio-organofertilization at different N-levels on: the degree of insect infestation by *Sitophilus oryzae* L. and *Rhizopertha dominica* F. J. Agric. Sci. Mansoura Univ., 25 (7): 4637-4645.
- Zidan, Z.H.; G. El-Saadany; Aziza Sharaby, Omar, E.A. and Shadia Abdel-Aziz (1994). Biological activity of essential oil of *Dodonaea viscosa* plant against the cotton leafworm *Spodoptera littoralis* 5<sup>th</sup> Conf. Agric. Ain Shams Univ., 2: 845-864.

**استجابة بعض النباتات الطبية للتلقيح المشترك بميكروبات تثبيت الأزوت وإذابة الفوسفات وعلاقة ذلك بالإصابة الحشرية فى المخزن**  
**هشام مصطفى الظن\* ، محمد نور الدين\*\* و سحر إبراهيم أحمد\*\*\***  
**\* قسم بحوث آفات المواد المخزنة - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقى - الجيزة**  
**\*\* قسم الميكروبيولوجى - معهد بحوث الأراضى والمياه والبيئة - مركز البحوث الزراعية - الجيزة**  
**\*\*\*قسم المبيدات - كلية الزراعة - جامعة كفر الشيخ**

أجريت تجربة لاختبار بذور أربعة نباتات طبية هى الحلبة، حبة البركة، حب الرشاد والخردل والتي سبق معاملتها قبل الزراعة فى الحقل بالتسميد الكيماوى المعتاد وكذا معاملتها بمخلوط من التسميد العضوى والحيوى المثبت للأزوت الجوى والمذيب للفوسفات وذلك لتقييم حساسية البذور الناتجة من النباتات المعاملة (١٦ معاملة) للإصابة بحشرة سوسة الأرز. - كذلك تم عمل حصر لتعداد الحشرات والأكاروس التى تصيب هذه النباتات الطبية المختبرة وعددها ١٦ معاملة وذلك لمدة عام تقريباً. - تم خلط هذه البذور بمعدل ١ ، ١٠٪ / وزن / وزن بحبوب القمح وتعريض هذا المخلوط لسوسة الأرز ودراسة نسبة الإصابة وتعداد الذرية الناتجة والنسبة المئوية للموت.

- أظهرت الدراسة أن نوع السماد ومعدلاته قد أثرت على نسبة الإصابة في حبوب القمح تحت الدراسة وقد يرجع ذلك إلى تغير في التركيب الكيماوى لبذور النباتات الطبية نتيجة المعاملة بالتسميد العضوى والحيوى مما قد يكون له تأثير على نسبة الإصابة في حبوب القمح.
- تم استخدام مبيد الأكتيليك كأحد المبيدات الحشرية الموصى بها فى مكافحة حشرات المواد المخزونة على سبيل المقارنة.
- أظهرت النتائج أن مبيد الأكتيليك كان أقوى المواد المستخدمة فى التأثير على سوسة الأرز حيث أدى إلى موت ١٠٠% للحشرات المعاملة وكذا أدى الي نسبة خفض ١٠٠% فى التعداد أو الذرية الناتجة وكانت نسبة الإصابة للحبوب المعاملة أقرب للصفر.
- لذلك نوصى بخلط حبوب القمح المخزونة مع بذور الحلبة بنسبة ١٠% حلبة بغرض تقليل الإصابة الحشرية لحبوب القمح وتقليل استخدام المبيدات الكيماوية لتحسين القيمة الغذائية للدقيق الناتج بعد اجراء التجارب التأكيدية مستقبلاً.

#### قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة  
كلية الزراعة – جامعة كفر الشيخ

أ.د / على عبد الهادى  
أ.د / عطيه يوسف قريطم