# Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 14 (4): 158-167, 2015
ISSN 1812-5387


CrossMark click for updates

ansinet
Asian Network for Scientific Information

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ppj.2015.158.167

# Effect of some Treatments on Seed Health and Viability of Soybean

E.A.M. Ibrahim
Department of Seed Technology Research, Field Crops Research Institute, ARC, Giza, Egypt

ARTICLE INFO
*Article History:*
Received: June 30, 2015
Accepted: August 07, 2015

Corresponding Author:
E.A.M. Ibrahim
Department of Seed Technology Research, Field Crops Research Institute, ARC, Giza, Egypt

ABSTRACT
This study was carried out in order to evaluate effect of treating soybean seed (*Glycin max* L.) as seed coating with fungicidal (Vetavax-thiram 200), seed dressing with biotic (Bio-arec, Bio-zeid) and seed soaking with abiotic (Salicylic acid) in controlling fungal diseases and improving seed and seedlings vigor of cultivars (Giza 21, G.35, G.111, G.22, G.82, Klark, Crawford) . The obtained results showed that, *Alternaria* spp., *Aspergillus* spp., *Cercospora kikuchii*, *Diaporthe sojae*, *Fusarium moniliforme*, *F. semitectum*, *F. solani*, *F. oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Penicillium* spp., were associated with soybean seed with different percentage. All treatments gave high significant $p \leq 0.05$ protection against seed-borne fungi in seed and seedling health evaluation test when applied seed treatments compared with control. Vitavax-200 was the most effective where lead to eliminated the mentioned fungi completely and improve of seed and seedling vigor characters. Giza 21 cv. recorded the less value borne of total fungi as compared of cultivars. Biotic reduced numbers of fungi and gave protection against pathogenic fungi and increased values seed and seedling vigor of soybean cultivars. Giza 82 cv. showing better seed and seedling vigor and less seed infection by pathogenic fungi in seedling health evaluation test. *Fusarium moniliforme* has been widely and most frequently with seed rot followed by *F. semitectum*. *Fusarium solani* was responsible for greater proportion of abnormal seedlings followed by *F. oxysporum*. Abiotic salicylic acid played important role in enhancing seed vigor, increased seedling length, dry weight of seedlings, reduced number of total fungi on seed and diseases incidence in seedlings (abnormal seedlings, seed rot).

**Key words:** Soybean, seed-borne fungi, vigor, fungicide, biotic, abiotic

## INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important oil seed crops in the world. Every population of soybean seeds is a potential carrier of various microorganisms, including fungi, bacteria and viruses (Sinclair and Shurtleff, 1975), also they found that *Diaporthe sojae* is one of the most important fungi lowering seed quality and affecting germination in soybean, *Aspergillus* spp., *Cercospora kikuchii* and *Macrophomina phaseolina* are the others among fungi contributing to lower seed quality in soybean. Medic-Pap *et al*. (2007) isolated pathogens fungi genera *Diaporthe*, *Fusarium* and facultative parasites, *Alternaria*, *Aspergillus* and *Penicillium* on soybean seeds. *Alternaria* spp., *Cercospora kikuchii*, *Fusarium* spp. *Penicillium* spp., *Aspergillus* spp. and *Sclerotinia* spp., lead to quality problems, deterioration and germination failure of soybean seeds (Gally, 2006). Goulart (2000) detected that *F. semitectum*, *Phomopsis* and *Cercospora kikuchii* with high levels on soybean seed samples. Pioli *et al*. (1997) isolated pathogens fungi in soybean seeds, *Alternaria* spp., *Cercospora kikuchii*, *Fusarium* spp., *Phomopsis* spp., *Rhizoctonia solani*, *Curvularia* spp. and *Nigrospora* spp., also they found correlations significant between each pathogen and germination percentage. *Phomopsis* spp. and *Fusarium* spp., were predominant in soybean seeds, Also *Phomopsis* sp., *Fusarium* spp., *Colletotrichum* spp., *Cercospora* spp., *Alternaria* spp., *Aspergillus* spp. and *Penicillium* spp., were identified by Braccini and Dhingra (1996) on soybean seeds. The pathogenic fungal species most frequently observed were *Phomopsis* spp., *F. moniliforme*, *F. semitectum* and *Fusarium* spp., while found *Sclerotinia scleroliorum*, *Macrophomina phaseolina*, *Botrytis cinerea* and *F. oxysporum* were detected at low percentages in soybean seed (Conca *et al*., 1996). Wilson *et al*. (1995) noticed that fungal damage caused by pathogens of *Fusarium, Cercospora and Phomopsis* can have a devastating impact on physical quality and seed composition to assess potential utility of highly damaged of soybean where found positive correlation between fungal damage and both protein and oil concen. *Aspergillus flavus*, *A. niger*, *F. oxysporum* and other unidentified fungi were observed abnormal seedlings with high percentage over 25% infection (Singh *et al*., 1995). Anwar *et al*. (1995) found that field fungi causing reduction of seed germination or seedling emergence were *Alternaria* spp., *Cercospora kikuchii*. *Fusarium moniliforme*, *F. oxysporum*, *F. solani* and *M. phaseolina* caused root diseases and damping off, while *A. alternata*, *C. kikuchii*, *Curvularia lunata*, *Phomopsis sojae* and *Stemphylium botryosum* caused foliage and pod disease. Incidence of *Alternaria* leaf spot (*A. alternate*), anthracnose (*Colletotrichum dematium*), *Cercospora* leaf spot (*C. kikuchii*), pod rot and blight (*F. semitectum* and *C. kikuchii*) and *Rhizoctonia* aerial blight (*R.. solani*) was higher in early sown cultivar compared with late sown, seed treated with thiram gave consistently higher seedling emergence than the untreated seed irrespective of sowing dates (Rahman *et al.*, 1995). Roy and Ratnayake (1997) found that *Fusarium semitectum* in seeds was negatively correlated with seed germination on agar and with seedling emergence in the greenhouse and reduced root volume, shoot dry weight of seedlings, also they found that seedling emergence was reduced to greater extent poor-quality seeds were inoculated with conidia of *F. semitectum* compared with high-quality of soybean seeds. Hamman *et al*. (1996) observed that germination percentage was reduced due to the effect of seed-borne pathogens, which resulted in relatively large numbers of dead seeds, abnormal seedlings and damaged seed coats, also found highly significant correlations between the number of dead seeds and the incidence of fungal infection and between the number of abnormal seedlings, seed coat damage of soybean seeds. Wahid *et al*. (1995) found that seed treatment soybean with Vitavax gave 100% inhibited growth of *F. moniliforme*, *F. solani* and *F. oxysporum* and improved germination. Seed treatment of soybean with thiram (0.3%) resulted in the highest percentage germination (Charjan and Tarar, 1992). Soybean seeds were treated with carbendaxim+thiram (1+1) at 0.3% eradicated *F. oxysporum*, *F. semitectum*, *M. phaseolina*, *A. flavus* and *A. alternate* and increased germination in the filed (Singh, 1997). Sonavane *et al*. (2011) showed that seed treatment of soybean with fungicidal (thiram+carbendazim) were significantly superior compared to the biological control agents (*Trichoderma viride*), where lead to lowest seed mycoflora, improved seed germination and seedling vigor index. Saikia *et a*l. (2003) reported that salicylic acid stimulated systemic resistance in chickpea against *Fusarium* wilt and reduced the disease 23-40%. Morsy *et al*. (2011) found that Bio-arec (*Bacillus megaterium*), Bio-zeid (*Trichoderma hamatum*) and abiotic salicylic acid lead to provide protection against alfalfa downy mildow, rust, root rot and wilt disease when applied as spry treatment or seed soaking. Biotic and abiotic lead to improving seed viability and reduction of fungal diseases as a safe way recommended in controlling of onion seeds (Ibrahim and Kishk, 2014). This study effect of seed coating with fungicide (Vitavax-thiram), seed dressing with biotic (Bio-arec, Bio-zeid) and seed soaking (salicylic acid) on seed, seedling vigor and control of seed-borne fungi on seed and seedling of soybean at Seed Pathology Laboratory, Seed Technology Research Unit, Mansoura, Egypt.

## MATERIALS AND METHODS

**Seed materials:** In the present study on samples of soybean from Department of Legumes Crops Research, Field Crops Research Institute, Agriculture Research Center, Egypt. Naturally infected seeds of soybean seven cultivars cvs Giza 21, G.35, G.111, G.22, G.82, Klark, Crawford used for this work in season 2014 for two times. Seed were surface sterilized in 1% sodium hypochlorite solution for 3 min, thin rinsed with sterilized water and air dried.

### Seed treatments

**Fungicide treatment (seed coating):** Soybean seed were used in this experiment for each cultivar were treated with Vitavax-Thiram 200 (carboxin 37.5+thiram 37.5%) at rate 2 g $kg^{-1}$ seeds and added the polymer coating 10 mL $kg^{-1}$ were taken in 100 mL dry flasks on a mechanical shaker for about 20 min tell the seeds are uniformly coated, later the treated seeds were spread on a sheet under shade and dried completely. The dried seeds were used for sowing.

**Biocides treatment (seed dressing):** Two different biological preparations namely Bio-arec (*Bacillus megaterium*) $25\times10^8$ CFU $g^{-1}$ and Bio-zeid (*Trichoderma hamatum* or *album*) $10\times10^8$ spore $g^{-1}$ obtained from Biological Control Unit, Plant Pathology Institute preparation contains active ingredient biocides to the recommendations of the Ministry of Agriculture, Egypt, were used at rate 5 g $kg^{-1}$ seed of soybean cultivars under study plus 3 mL sterile water, all were mixed properly as seed dressing treatment and air dried for 30 min on sterile tray to enable the seeds to absorb the biocides and used for sowing.

**A biotic treatments (seed soaking):** Soybean seeds for each cultivar were completely dipped in the solution of salicylic acid concentration 10 mM for 12 h. The treated seeds were dried for 24 h at room temperature and used for sowing.

**Seed health evaluation (blotter test):** Two hundred of soybean seeds were used in this experiment for each cultivar and treat were plated on three layers of filter paper will soaked in distilled water in eight replicates (25 seeds/Petri dish 12 cm). Other two hundred seeds un treated as a control were incubated at 25°C±2 for seven days under alternating cycles of 12 h near ultraviolet (NUV) light and darkens. Percentage of fungi under investigation were calculated by stereo-binocular microscope (Wild Heerbrugg 6.3-32X) and recorded to the following equation:

$$\text{Fungal (\%)} = \frac{n1}{n2} \times 100$$

where, n1 the seeds with fungal growth and n2 the number of treated seeds.

**Seed and seedling vigor evaluation in pots:** One hundred seeds for each treatment and cultivar were planted in 20 cm diameter pots containing sterilized sand in 4 replicates at the rate 25 seeds/pot and other untreated seeds were used as check. Pots were incubated in a growth chamber (Seedburo Equipment Company, USA) for 14 days at 25°C day and night lengths 15/9 h to study the following characters:

- **Germination percentage (G%) (normal seedlings):** It was calculated by counting only normal seedlings (ISTA., 1999) rules:

$$\text{MGP} = \frac{n1 - n2}{n1} \times 100$$

  where, MGP is the mean percentage, n1 is number of treated seed plated and n2 the number of abnormal seedlings plus died seeds
- **Speed Germination Index (SGI):** It was calculated as described in the (AOSA., 1983) by the following equation:

$$\text{SGI} = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

- At the final count, five normal seedlings from each replicate were randomly taken to measure seedling characters, seedling length (cm), dry weight (g) of seedlings (ISTA., 1999) rules:

Seedling Vigor Index (SVI) = MGB×seedling dry weight

**Seedling health evaluation:** The seedling test were separated into abnormal seedlings, seed rot and the causal fungi examined using a stereo-scopic microscope and recorded percentage of fungi as described in the technical bulletin on seed-borne diseases (Agarwal *et al*., 1989; Ellis, 1980):

$$\text{Fungal infection (\%)} = \frac{\text{No. of fungal infection on abnormal seedlings or seed rot}}{\text{Total of abnormal seedlings or seed rot}} \times 100$$

**Statistical analysis:** The observed data was statistically analyzed as the technique of analysis of variance ANOVA of the randomized complete block design as mentioned by Gomez and Gomez (1984). The means were compared using the Least Significant Differences (LSD). Statistical analysis was performed using analysis of variance technique ANOVA by means of MSTAT-C" computer software package.

## RESULTS

**Identification of soybean seed-borne fungi (seed health evaluation):** Seven cultivars of soybean seed samples were tested for using the standard blotter method seedlings (ISTA., 1999) rules, i.e. Giza 21, G. 35, G.111, G. 22, G.82, klark, Crawford. Eleven seed-borne fungi were identified as *Alternaria* spp., *Aspergillus* spp., *Cercospora kikuchii*, *Diaporthe sojae*, *Fusarium moniliforme*, *F. semitectum*, *F. solani*, *F. oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Penicillium* spp., associated of soybean seed. Data presented in (Table 1) revealed that the highest frequency percentage was recorded by *Aspergillus* spp. (7.5%) on cv. G.22, *Penicillium* spp. (5.6%) on cv. G.82, while *C. kikuchii*, *F. moniliforme* and *F. solani* recorded (3.5%) on cvs G. 22, G. 111, G. 111 and *F. oxysporum* (3,2%) on cv. Crawford as pathogenic fungi, also cv. G. 22 was the highest of the total fungi (28.4) followed by cvs. G. 35, G. 82, G. 111, Clark, Crawford and G. 21 was the less (18.4).

Also, data presented in Table 1 indicated that Vitavax-200 used for seed coating lead to eliminate of most fungi, meanwhile Bio-arec as seed coating reduced *Aspergillus* spp., from 13.7-4%, *C. kikuchii* from 4.4-1.1%, *D. sojae* from 4.2-0.6% and *F. moniliforme* from 8-1.5%, Also, Bio-zeid was able to reduced *F. oxysporum* from 5.9-1.3%, *M. phaseolina* from 1.7-0%. Salicylic acid as seed soaking showed least of fungal infection percentage such as *Penicillium* spp., from 12-4.9%, *F. solani* from 3.8-0%, *F. semitectum* from 6.5-1.2%, *F. moniliforme* from 8-0.4% and total fungi from 67-13. Generally, effect all treatments were high significant with fungal infection percentage for each fungi.

Interaction between cultivars, treatments and the effect on seed-borne fungi associated of soybean seed (Table 2), indicated that on seed of cv. G. 21, *Aspergillus* spp., recorded the highest frequency percentage (16%) followed by *Penicillium* spp. (9.3%), *D. sojae* (5.3%), *F. oxysporum*

Table 1: Frequency of occurrence of seed-borne fungi associated with cvs soybean after seed treatment by Blotter method

| Fungi | *Alternaria* ssp. | *Aspergillus* spp. | *Cercospora kikuchii* | *Diaporthe sojae* | *Fusarium moniliforme* | *F. semitectum* | *F. solani* | *F. oxysporum* | *M. phaseolina* | *R. solani* | *Penicillium* spp. | *T. fungi* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivars** | | | | | | | | | | | | |
| Giza 21 | 0.5 | 5.1 | 0.5 | 1.8 | 2.4 | 1.1 | 0.2 | 1.3 | 0.0 | 1.3 | 4.2 | 18.4 |
| G.35 | 1.6 | 3.7 | 0.5 | 0.5 | 2.1 | 2.1 | 1.3 | 2.4 | 0.5 | 1.6 | 5.3 | 21.6 |
| G.111 | 0.8 | 4.8 | 1.6 | 0.0 | 3.5 | 1.1 | 3.5 | 0.8 | 0.5 | 0.2 | 4.3 | 21.1 |
| G.22 | 4.0 | 7.5 | 3.5 | 0.8 | 2.6 | 2.4 | 0.5 | 1.0 | 1.0 | 0.3 | 5.3 | 28.4 |
| G.82 | 0.8 | 4.7 | 1.3 | 1.0 | 2.1 | 1.6 | 0.5 | 2.9 | 1.3 | 0.3 | 5.6 | 21.6 |
| Klark | 0.0 | 5.5 | 0.3 | 2.1 | 1.1 | 2.4 | 0.0 | 2.1 | 1.3 | 0.8 | 4.0 | 19.6 |
| Crawford | 0.0 | 3.7 | 1.3 | 1.6 | 1.1 | 1.8 | 0.5 | 3.2 | 0.0 | 0.5 | 4.5 | 18.2 |
| LSD (5%) | - | - | 1.6 | - | - | - | 1.7 | | - | - | - | |
| **Treatments** | | | | | | | | | | | | |
| Control | 3.4 | 13.7 | 4.9 | 4.2 | 8.0 | 6.5 | 3.8 | 5.9 | 1.7 | 2.0 | 12.0 | 67.0 |
| Vitavax-200 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 | 0.0 | 0.0 | 0.0 | 11.0 |
| Bio-arec | 0.8 | 4.0 | 1.1 | 0.6 | 1.5 | 0.7 | 0.4 | 1.3 | 0.2 | 0.4 | 4.0 | 15.0 |
| Bio-zeid | 0.6 | 2.1 | 0.0 | 1.0 | 0.8 | 1.5 | 0.6 | 1.3 | 0.0 | 0.8 | 2.5 | 11.0 |
| SA | 0.8 | 3.6 | 0.4 | 0.0 | 0.4 | 1.2 | 0.0 | 1.2 | 0.0 | 0.4 | 4.9 | 13.0 |
| LSD (5%) | 1.5 | 2.1 | 1.2 | 1.1 | 1.8 | 1.6 | 1.1 | 2.1 | 0.6 | 1.1 | 2.2 | |

Test was carried out using blotter technique, Two hundred seeds were tested, Incubation was carried out at 25°C±for 7 days (ISTA., 1999), SA: Salicylic acid, LSD: Least significant difference

(5.3%) as control. Cultivar G. 35 revealed that *Penicillium* spp. was (13.3%) followed by *Aspergillus* spp. (12%) thin *F. moniliforme*, *F. semitectum* (6.6%) and *F. solani* (5.3%). *Fusarium solani* was 13% and both *Aspergillus* spp. and *F. moniliforme* were (12%), while *C. kikuchii* and *F. semitectum* (5.3%) on cv. G. 111as control. On cv. G. 22 recorded by blotter test, *Aspergillus* spp. and *Penicillium* spp. 24 and 14.6%, respectively, *C. kikuchii* was (13.3%) and *F. semitectum* (8%). *Aspergillus* spp., was (13%), *Penicillium* spp. (14.6%), *F. oxysporum* (9.3%), *F. moniliforme* (8%), *F. semitectum* (6.7%) and *C. kikuchii* (5.3%) on seed of cv. G. 82. Also, results pointed out that *Aspergillus* spp. and *Penicillium* were the highest infection percentage (12%) followed by *F. semitectum* (9.3%), while *D. sojae* and *F. oxysporum* were (6.6%) on seed of cv. Klark. Crawford cultivar recorded with *Penicillium* spp. (12%), *F. oxysporum* 10.6 and 6.6% both *D. sojae* and *Aspergillus* spp., respectively, *F. moniliforme* and *F. semitectum* were (5.3%). On the other hand, cv. G. 22 recorded the highest number of total fungi (98.6) followed by cv. G. 82 (68.8), cv. G111 (68.4), cv. G. 35 (62.3), Klark (57.2), Crawford (55.7) and cv. G. 21 (54.4) as control. Data in Table 2 indicated that the fungicide (Vitavax-200) was effective in almost, eliminated of seed-borne fungi to all cultivars used in this experiment, while biotic (Bio-arec) reduced total fungi from 98.6-26.4, (Bio-zeid) to 14.6, a biotic (SA) reduced total fungi to 5.3 on seed of cv. G. 22. Also, results showed that *Penicillium* spp. recorded the highest number of the total fungi (167.1) and the more frequency on cvs soybean seed as survey compared with other fungi, followed by *Aspergillus* spp. (163.3) thin *F. moniliforme* (74.1), *F. oxysporum* (68.5), *F. semitectum* (63.3), *C. kikuchii* (44.9), *Alternaria* spp. (42.5), *D. sojai* (39.6), *F. solani* (32.8), *R. solani* (25) and *M. phaseolina* (14.1).

Effect of cultivars and seed treatments under study on seed and seedling vigor of soybean Table 3 shows that cv. Giza 82 recording the highest value of Mean Germination Percentage (MGP) (88%), Speed Germination Index (SGI) (8.4) and Seedling Length (SL) (24.5 cm), while cv. G. 21 was the less value of MGP (80%), SL (18.5 cm) and dry weight (DG) (1.2 Gm.) as compared cultivars. On the other hand, effect of treatments were high significant to characters vigor, seed soaking with Salicylic Acid (SA) showed better germination (MGP) (91%), SPI (8.6), SL (30 cm), DW (1.7 Gm.) and gave the highest value of seedling vigor index followed by Vitavax-200 gave (89%), MGP, Bio-zeid (88%) MGP and Bio-arec (87%) MGP as compared with control (68%).

Seedling health evaluation, abnormal seedlings and seed rot infection with pathogenic fungi tabulated in Table 4. Abnormal seedlings recorded the highest Percentage of Fungal Infection (PFI) by *D. sojae*, *F. oxysporum* and *R. solani* were (9%) to cvs Klark, G. 35 and G. 111 as control, respectively, while *F. solani* recorded 7 and 8% with cv. G. 111 and Klark, respectively. *Fusarium semitectum* gave percentage of fungal infection (5%) with cvs G. 35 and G. 21, while *C. kikuchii* recorded (4%) with cvs G. 21, G. 35 and G. 82. *Macrophomina phaseolina* gave PFI with abnormal seedlings of cvs G. 111, Klark and G. 21 5, 4 and 3%, respectively as control. Seed rot type gave to *F. semitectum* and *F. moniliforme* the highest value (5%) with cv. G. 111 and (4%) with cv. Klark, respectively. Generally, on seed of cvs soybean found lowering of MGP due to association high percentages of pathogenic fungi for seed and seedlings as *C. kikuchii*, *D. sojae*, *F. moniliforme*, *F. semitectum*, *F. solani*, *F. oxysporum*, *M. phaseolina* and *R. solani*.

Table 5 showed that the effect of treatments on percentage of fungi and relation to abnormal seedlings and seed rot, *F. solani* was the more fungi associated of abnormal seedlings with (9%) followed by *F. oxysporum* (6.8%) thin *R. solani* (6%), *D. sojae* (5.2%), *F. semitectum* (5%), *C. kikuchii* (4.2%) and *M. phaseolina* (3%) as control, respectively (Table 5). Also *F. moniliforme* has been widely and most frequently with seed rot where recorded (11.5%) followed by *F. semitectum*

Table 2: Interaction between cultivars, treatments and the effect on seed borne fungi associated of soybean seed

| Cultivar and treatment | *Alternaria* spp. | *Aspergillus* spp. | *Cercospora kikuchii* | *Diaporthe sojae* | *Fusarium moniliforme* | *F. semitectum* | *F. solani* | *F. oxysporum* | *M. phaseolina* | *R. solani* | *Penicillium* spp. | Total fungi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Giza 21** | | | | | | | | | | | | |
| Control | 0.0 | 16.0 | 2.6 | 5.3 | 8.0 | 4.0 | 0.0 | 5.3 | 0.0 | 4.0 | 9.3 | 54.4 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bio-arec | 0.0 | 4.0 | 0.0 | 1.3 | 2.6 | 0.0 | 0.0 | 1.3 | 0.0 | 1.3 | 2.6 | 13.1 |
| Bio-zeid | 0.0 | 2.6 | 0.0 | 2.6 | 0.0 | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 2.3 | 8.8 |
| SA | 2.6 | 2.6 | 0.0 | 0.0 | 1.3 | 1.3 | 0.0 | 0.0 | 0.0 | 1.3 | 8.0 | 17.1 |
| **Giza 35** | | | | | | | | | | | | |
| Control | 4.0 | 12.0 | 2.6 | 2.7 | 6.6 | 6.6 | 5.3 | 2.6 | 2.6 | 4.0 | 13.3 | 62.3 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 5.3 | 0.0 | 0.0 | 0.0 | 5.3 |
| Bio-arec | 1.3 | 1.3 | 0.0 | 0.0 | 1.3 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | 9.2 |
| Bio-zeid | 0.0 | 0.0 | 0.0 | 0.0 | 2.6 | 2.6 | 1.3 | 4.0 | 0.0 | 2.6 | 4.0 | 17.1 |
| SA | 2.6 | 5.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.3 | 5.3 | 14.5 |
| **Giza 111** | | | | | | | | | | | | |
| Control | 4.0 | 12.0 | 5.3 | 0.0 | 12.0 | 5.3 | 13.0 | 2.6 | 3.6 | 0.0 | 10.6 | 68.4 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bio-arec | 0.0 | 4.0 | 1.3 | 0.0 | 1.3 | 0.0 | 2.6 | 0.0 | 0.0 | 0.0 | 2.6 | 11.8 |
| Bio-zeid | 0.0 | 0.0 | 0.0 | 0.0 | 2.6 | 0.0 | 1.3 | 1.3 | 0.0 | 1.3 | 1.3 | 7.8 |
| SA | 0.0 | 8.0 | 1.3 | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 | 17.3 |
| **Giza 22** | | | | | | | | | | | | |
| Control | 12.0 | 24.0 | 13.3 | 4.0 | 12.0 | 8.0 | 2.7 | 4.0 | 4.0 | 0.0 | 14.6 | 98.6 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bio-arec | 4.0 | 6.6 | 4.0 | 0.0 | 1.3 | 1.3 | 0.0 | 1.3 | 1.3 | 1.3 | 5.3 | 26.4 |
| Bio-zeid | 4.0 | 4.0 | 0.0 | 0.0 | 0.0 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | 14.6 |
| SA | 4.0 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.7 | 9.3 |
| **Giza 82** | | | | | | | | | | | | |
| Control | 4.0 | 13.0 | 5.3 | 4.0 | 8.0 | 6.7 | 2.6 | 9.3 | 1.3 | 0.0 | 14.6 | 68.8 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bio-arec | 0.0 | 2.6 | 1.3 | 0.0 | 2.6 | 2.3 | 0.0 | 1.3 | 0.0 | 0.0 | 4.0 | 14.1 |
| Bio-zeid | 0.0 | 4.0 | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 2.6 | 0.0 | 1.3 | 2.7 | 11.9 |
| SA | 0.0 | 3.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.3 | 0.0 | 0.0 | 6.7 | 11.6 |
| **Klark** | | | | | | | | | | | | |
| Control | 0.0 | 12.0 | 1.3 | 6.6 | 4.0 | 9.3 | 0.0 | 6.6 | 1.3 | 4.0 | 12.0 | 57.1 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bio-arec | 0.0 | 6.6 | 0.0 | 1.3 | 1.3 | 0.0 | 0.0 | 2.6 | 0.0 | 0.0 | 5.3 | 17.1 |
| Bio-zeid | 0.0 | 2.6 | 0.0 | 2.6 | 0.0 | 2.7 | 0.0 | 1.3 | 0.0 | 0.0 | 1.3 | 10.5 |
| SA | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.3 | 2.6 |
| **Crawford** | | | | | | | | | | | | |
| Control | 0.0 | 6.6 | 4.0 | 6.6 | 5.3 | 5.3 | 2.7 | 10.6 | 0.0 | 2.6 | 12.0 | 55.7 |
| Vitavax | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.6 | 0.0 | 0.0 | 0.0 | 2.6 |
| Bio-arec | 0.0 | 2.6 | 1.3 | 1.3 | 0.0 | 1.3 | 0.0 | 2.6 | 0.0 | 0.0 | 4.0 | 13.1 |
| Bio-zeid | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 2.7 | 0.0 | 0.0 | 0.0 | 0.0 | 2.7 | 6.7 |
| SA | 0.0 | 2.6 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | 7.9 |
| Total fungi | 42.5 | 163.8 | 44.9 | 39.6 | 74.1 | 63.3 | 32.8 | 68.5 | 14.1 | 25.0 | 167.1 | 735.7 |
| LSD (5%) | 3.8 | 5.4 | 3.2 | - | - | - | 3.0 | - | - | - | - | |

Table 3: Effect of cultivars and seed treatments on seed and seedling vigor of soybean in Pots

| | Characters | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | G (%) | SG | SL (cm) | AS | SR | DW (g) | SVI |
| **Cultivars** | | | | | | | |
| Giza 21 | 80.0 | 8.1 | 18.5 | 14 | 6 | 1.2 | 96.0 |
| Giza 35 | 83.0 | 7.7 | 24.0 | 13 | 4 | 1.3 | 107.9 |
| Giza 111 | 84.0 | 7.6 | 20.3 | 11 | 5 | 1.3 | 109.2 |
| Giza 22 | 87.0 | 8.0 | 23.0 | 9 | 4 | 1.3 | 113.1 |
| Giza 82 | 88.0 | 8.4 | 24.5 | 10 | 2 | 1.3 | 114.4 |
| Klark | 85.0 | 8.2 | 24.4 | 11 | 4 | 1.4 | 119.0 |
| Crawford | 86.0 | 7.9 | 21.1 | 11 | 3 | 1.4 | 120.4 |
| LSD (5%) | - | - | 2.3 | - | - | - | - |
| **Treatments** | | | | | | | |
| Control | 68.0 | 7.9 | 17.0 | 24 | 8 | 1.1 | 74.8 |
| Vetavax-200 | 89.0 | 7.4 | 19.1 | 9 | 2 | 1.1 | 97.9 |
| Bio-arec | 87.0 | 7.9 | 23.0 | 10 | 3 | 1.3 | 113.1 |
| Bio-zeid | 88.0 | 8.0 | 22.2 | 9 | 3 | 1.3 | 114.4 |
| Salicylic acid | 91.0 | 8.6 | 30.2 | 4 | 5 | 1.7 | 154.7 |
| LSD (5%) | 3.3 | 0.4 | 3.4 | 3.6 | 2.2 | 0.2 | 0.2 |
| Interaction AXB LSD (5%) | 9.7 | - | - | - | - | - | - |

G (%): Mean germination percentage, SG: Speed germination, SL: Seedling length, AS: Abnormal seedlings, SR: Seed rot, DW: Dry weight, SVI: Seedling vigor index, LSD: Least significant difference

thin *Aspergillus* spp. and *Penicillium* spp. On the other hand Vitavax-200 lead to elimination fungi such as *C. kikuchii*, *D. sojae*, *F. moniliforme*, *F. solani*, *M. phaseolina* and *R. solani* in seed rot, Bio-arec lead to less number of infection by pathogenic fungi compared to the control as *F. oxysporum* from 6.8-2.6% and *R. solani* from 6-1% in abnormal seedlings. Bio-zeid less seed infection of *F. moniliforme* from 11.5-1.4%, also *F. semitectum* from 6.7- 2% with seed rot. Salicylic acid lead to eliminating and/or reducing number of pathogenic fungi on both abnormal seedlings and seed rot as compared with control and other treatments.

Table 6 on seed of Giza 21 show that *F. semitectum* caused the highest infection percentage of abnormal seedlings (3.4%), *F. solani* (4.1%) on seed rot. Also, on cv. G. 35 recorded *F. moniliforme* the highest infection on seed rot (6.8%) followed by G. 22 (5.4%), while *C. sojae* was (2.3%) on abnormal seedlings with cv.G.35. Giza 82 cv. was the less cultivars effective with total fungi on abnormal seedlings and seed rot as compared other cultivars, on the other hand cv. G. 21 was the more cultivars as seed-borne of total fungi in abnormal seedlings and seed rot.

Table 4: Percentage of fungal infection in abnormal seedlings and seed rot after seed treatment of soybean cultivars in pots

| | Cultivars | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Giza 21 | | | | | Giza 35 | | | | | Giza 111 | | | | | Giza 22 | | | | |
| Treatments | Control | Vetavax | Bio-arec | Bio-zeid | SA | Control | Vetavax | Bio-arec | Bio-zeid | SA | Control | Vetavax | Bio-arec | Bio-zeid | SA | Control | Vetavax | Bio-arec | Bio-zeid | SA |
| NS | 68 | 83 | 79 | 83 | 89 | 59 | 92 | 83 | 89 | 91 | 59 | 87 | 88 | 93 | 93 | 75 | 93 | 89 | 85 | 92 |
| AS | 23 | 13 | 5 | 12 | 5 | 35 | 7 | 13 | 9 | 4 | 28 | 12 | 8 | 5 | 2 | 18 | 7 | 8 | 10 | 3 |
| SR | 9 | 4 | 16 | 5 | 6 | 6 | 1 | 4 | 2 | 5 | 13 | 1 | 4 | 2 | 5 | 7 | 0 | 3 | 5 | 5 |
| ***Alternaria* spp.** | | | | | | | | | | | | | | | | | | | | |
| AS | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| SR | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| ***Aspergillus* spp.** | | | | | | | | | | | | | | | | | | | | |
| AS | 4 | 3 | 0 | 4 | 3 | 3 | 2 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 1 |
| SR | 3 | 1 | 0 | 3 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| ***C. kikuchii*** | | | | | | | | | | | | | | | | | | | | |
| AS | 4 | 0 | 0 | 0 | 0 | 4 | 1 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 3 | 3 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| ***D. sojae*** | | | | | | | | | | | | | | | | | | | | |
| AS | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 1 | 0 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***F. moniliforme*** | | | | | | | | | | | | | | | | | | | | |
| AS | 1 | 1 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SR | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 4 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 1 | 2 |
| ***F. semitectum*** | | | | | | | | | | | | | | | | | | | | |
| AS | 5 | 2 | 4 | 1 | 1 | 5 | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 3 | 1 | 0 |
| SR | 1 | 0 | 3 | 0 | 1 | 3 | 0 | 2 | 0 | 0 | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***F. solani*** | | | | | | | | | | | | | | | | | | | | |
| AS | 4 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 7 | 0 | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| SR | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| ***F. oxysporum*** | | | | | | | | | | | | | | | | | | | | |
| AS | 1 | 2 | 1 | 0 | 0 | 9 | 1 | 4 | 1 | 1 | 1 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 3 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| ***M. phaseolina*** | | | | | | | | | | | | | | | | | | | | |
| AS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| SR | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***R. Solani*** | | | | | | | | | | | | | | | | | | | | |
| AS | 1 | 0 | 0 | 1 | 0 | 4 | 0 | 1 | 4 | 0 | 9 | 0 | 0 | 1 | 0 | 5 | 0 | 3 | 3 | 0 |
| SR | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Penicillium* spp.** | | | | | | | | | | | | | | | | | | | | |
| AS | 3 | 2 | 0 | 5 | 1 | 1 | 0 | 0 | 0 | 3 | 1 | 2 | 4 | 0 | 1 | 1 | 5 | 0 | 0 | 1 |
| SR | 1 | 3 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 3 | 3 | 1 | 0 | 1 | 7 | 0 | 0 | 0 | 1 | 3 |

Table 4: Continue

| | Cultivars | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Giza 82 | | | | | Klark | | | | | Crawford | | | | |
| treatments | Control | Vetavax | Bio-arec | Bio-zeid | SA | Control | Vetavax | Bio-arec | Bio-zeid | SA | Control | Vetavax | Bio-arec | Bio-zeid | SA |
| NS | 77 | 91 | 91 | 91 | 91 | 67 | 91 | 84 | 91 | 94 | 72 | 87 | 96 | 85 | 91 |
| AS | 20 | 7 | 6 | 8 | 7 | 25 | 8 | 12 | 8 | 2 | 21 | 12 | 4 | 11 | 5 |
| SR | 3 | 2 | 3 | 1 | 2 | 8 | 1 | 4 | 1 | 4 | 7 | 1 | 0 | 4 | 4 |
| ***Alternaria* spp.** | | | | | | | | | | | | | | | |
| AS | 1 | 3 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| SR | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 3 | 1 | 0 | 0 | 0 |
| ***Aspergillus* spp.** | | | | | | | | | | | | | | | |
| AS | 1 | 1 | 0 | 2 | 1 | 0 | 8 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| SR | 1 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 3 |
| ***C. kikuchii*** | | | | | | | | | | | | | | | |
| AS | 4 | 0 | 1 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***D. sojae*** | | | | | | | | | | | | | | | |
| AS | 2 | 0 | 3 | 2 | 1 | 9 | 0 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 |
| SR | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***F. moniliforme*** | | | | | | | | | | | | | | | |
| AS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SR | 2 | 0 | 0 | 1 | 0 | 4 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| ***F. semitectum*** | | | | | | | | | | | | | | | |
| AS | 2 | 0 | 0 | 1 | 0 | 3 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***F. solani*** | | | | | | | | | | | | | | | |
| AS | 2 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 2 | 0 | 7 | 0 | 0 | 3 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***F. oxysporum*** | | | | | | | | | | | | | | | |
| AS | 5 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 7 | 0 | 2 | 1 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***M. phaseolina*** | | | | | | | | | | | | | | | |
| AS | 3 | 0 | 0 | 3 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***R. solani*** | | | | | | | | | | | | | | | |
| AS | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 0 |
| SR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Penicillium* spp.** | | | | | | | | | | | | | | | |
| AS | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 4 | 2 | 1 | 0 | 11 | 2 | 0 | 7 |
| SR | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 3 | 3 | 0 | 4 | 1 |

NS: Normal seedlings, plumlee and root well developed, AS: Abnormal seedlings, decay in shoot and root, primary root showing browning no branching or secondary development shoot usually appearing weak, SR: Seed rot, consisted of non-germinated seeds covered with hyphal growth of the tested (Guerrero *et al*.,1972)

Table 5: Effect of treatments on percentage of fungal infection and relation to percentage of abnormal seedlings and seed rot of soybean

| | Control (%) | | Vitavax-200 (%) | | Bio-arec (%) | | Bio-zeid (%) | | Salicylic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fungi | AS (n = 44) | SR (n = 36) | AS (n = 17) | SR (n = 7) | AS (n = 15) | SR (n = 23) | AS (n = 16) | SR (n = 13) | AS (n = 8) | SR (n = 21) |
| *Alternaria* spp. | 1.0 | 4.0 | 2.6 | 0.6 | 0.5 | 0.8 | 0.5 | 0.0 | 0.3 | 0.7 |
| *Aspergillus* spp. | 2.3 | 5.4 | 4.6 | 1.3 | 0.5 | 3.0 | 1.0 | 4.0 | 1.8 | 5.0 |
| *Cercospora kikuchii* | 4.2 | 0.7 | 0.3 | 0.0 | 2.3 | 0.0 | 2.0 | 0.0 | 0.5 | 0.0 |
| *Diaporthe sojae* | 5.2 | 0.0 | 0.5 | 0.0 | 2.3 | 0.0 | 2.0 | 0.0 | 0.3 | 0.6 |
| *Fusarium moniliforme* | 1.0 | 11.5 | 0.8 | 0.0 | 0.0 | 7.1 | 0.0 | 1.4 | 0.0 | 3.3 |
| *F. semitectum* | 5.0 | 6.7 | 1.0 | 1.4 | 2.8 | 3.3 | 1.3 | 2.0 | 0.2 | 0.6 |
| *F. solani* | 9.0 | 2.0 | 0.0 | 0.0 | 0.3 | 4.0 | 1.3 | 0.0 | 0.0 | 0.0 |
| *F. oxysporum* | 6.8 | 2.0 | 0.7 | 1.3 | 2.6 | 1.0 | 2.6 | 0.0 | 0.7 | 0.0 |
| *M. phaseolina* | 3.0 | 2.7 | 0.0 | 0.0 | 0.7 | 1.0 | 0.7 | 0.6 | 0.2 | 0.0 |
| *R. solani* | 6.0 | 0.0 | 0.0 | 0.0 | 1.0 | 2.0 | 2.8 | 0.0 | 0.0 | 0.0 |
| *Penicillium* spp. | 1.6 | 4.7 | 6.0 | 6.7 | 2.6 | 1.0 | 1.8 | 5.4 | 4.0 | 12.0 |
| Total fungi | 45.0 | 39.7 | 16.5 | 11.3 | 15.6 | 23.2 | 16.0 | 13.4 | 8.0 | 22.2 |

Table 6: Percentage of fungal infection and relation to abnormal seedlings, seed rot and cultivars of soybean

| | Giza 21 (%) | | G 35 (%) | | G 111 (%) | | G 22 (%) | | G 82 (%) | | Klark (%) | | Crawford (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fungi | AS (n = 15) | SR (n = 27) | AS (n = 18) | SR (n = 12) | AS (n = 14) | SR (n = 17) | AS (n = 12) | SR (n = 14) | AS (n = 13) | SR (n = 7) | AS (n = 14) | SR (n = 12) | AS (n = 14) | SR (n = 11) |
| *Alternaria* spp. | 0.8 | 0.7 | 0.5 | 0.0 | 1.3 | 0.0 | 0.3 | 0.7 | 1.3 | 0.7 | 0.5 | 2.7 | 0.3 | 3.4 |
| *Aspergillus* spp. | 3.6 | 5.4 | 1.3 | 2.0 | 1.3 | 2.7 | 0.5 | 0.7 | 1.3 | 2.7 | 2.6 | 3.4 | 0.3 | 2.7 |
| *Cercospora kikuchii* | 1.0 | 0.7 | 2.3 | 0.0 | 0.3 | 0.0 | 1.8 | 0.7 | 1.6 | 0.0 | 0.8 | 0.0 | 1.0 | 0.0 |
| *Diaporthe sojae* | 0.4 | 0.0 | 1.8 | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 2.0 | 0.7 | 2.9 | 0.0 | 1.6 | 0.0 |
| *Fusarium moniliforme* | 0.5 | 2.7 | 1.3 | 6.8 | 0.0 | 2.0 | 0.0 | 5.4 | 0.0 | 2.0 | 0.0 | 3.4 | 0.0 | 1.4 |
| *F. semitectum* | 3.4 | 3.4 | 2.3 | 3.4 | 0.5 | 4.7 | 1.8 | 2.0 | 0.9 | 0.0 | 1.3 | 0.7 | 0.3 | 0.0 |
| *F. solani* | 1.0 | 4.1 | 0.8 | 0.0 | 2.1 | 1.4 | 1.3 | 0.7 | 0.5 | 0.0 | 2.6 | 0.0 | 2.6 | 0.0 |
| *F. oxysporum* | 1.0 | 0.0 | 4.0 | 0.0 | 1.0 | 2.0 | 1.6 | 2.0 | 2.0 | 0.0 | 1.0 | 0.0 | 2.6 | 0.0 |
| *M. phaseolina* | 0.0 | 2.0 | 0.0 | 0.7 | 1.3 | 0.7 | 0.3 | 0.0 | 1.6 | 0.0 | 1.6 | 0.0 | 0.3 | 0.0 |
| *R. solani* | 0.5 | 2.0 | 2.3 | 0.0 | 2.6 | 0.0 | 2.9 | 0.0 | 0.3 | 0.0 | 0.5 | 0.0 | 0.8 | 0.0 |
| *Penicillium* spp. | 2.8 | 6.0 | 0.8 | 2.0 | 2.3 | 8.1 | 1.8 | 2.7 | 1.6 | 2.0 | 1.8 | 2.0 | 5.2 | 7.4 |
| Total fungi | 15.0 | 27.0 | 18.4 | 14.9 | 14.0 | 21.1 | 12.3 | 14.9 | 13.1 | 8.1 | 15.6 | 12.0 | 15.0 | 14.9 |

## DISCUSSION

Seed-borne fungal inocula are known to cause several levels of lowering seed quality and affecting germination in soybean (Sinclair and Shurtleff, 1975). The present study investigated 7 cultivars of soybean seed by blotter method and identification 11 species 8 genera, namely *Alternaria* spp., *Aspergillus* spp., *C. kikuchii*, *D. sojae*, *F. moniliforme*, *F. semitectum*, *F. solani*, *F. oxysporum*, *M. phaseolina*, *R. solani* and *Penicillium* spp. (Medic-Pap *et al*., 2007; Goulart, 2000; Pioli *et al*., 1997). These results point to *Aspergillus* spp., was the more frequency fungi on seeds followed by *Penicillium* spp., both it from saprophyte fungi but found that cases low percentages in normal seedlings and deterioration in vigor seed, Krishnamurty and Raveesha (1996) indicated that *Aspergillus values* was the frequent occurrence of orange-yellow discolored which were highly distorted, shriveled and smaller than normal seeds and isolated from seed coats, cotyledons and internal tissues of the discolored seeds. On the other hand, data obtained showed that *C. kikuchii*, *D. sojae*, *Fusarium* spp., *M. phaseolina* and *R. solani* as pathogenic fungi were observed on abnormal seedlings and seed rot with high percentage and causing reduction of seed germination or seedling emergence. These results were in harmony with those reported by Anwar *et al*. (1995), Singh *et al*. (1995), Roy and Ratnayake (1997) and Hamman *et al*. (1996). *Fusarium* spp., observed associated with seed rot and abnormal seedlings and produce rotten lesions on cotyledons and hypocotyls also plumlee soft rot of soybean seedlings (Gally *et al*., 1998). *Fusarium oxysporum* externally appeared shrunken, slightly irregular in shape, often with cracks in the seed coat with light to dark pink discolored areas over most of the infected seed surface, seeds with severe symptoms did not germinate, hypha were found spread over the surface of the seed coat producing macro-and micro conidia and within the hilum region (Velicheti and Sinclair,1991). *Fusarium* wilt caused by *F. oxysporum* is one of the major diseases of soybean, the fungus invades plant vascular tissues and induces sever wilting of the foliage by blocking xylem transport and impeding the movement of water, the pathogen is both seed and soil borne. Haikal (2008) soaked seeds of soybean with culture filtrates detected that percentage seed germination and seedling growth decreased with increase in filtrate concentration, filtrate age and pre-soaking time in all the fungal filtrates *A. niger*, *F. culmorium*, *Penicillium* spp. and *R. solani*. *Macrophomina phaseolina* as seed transmission causing charcoal rot, grey to black fungal growth were observed on the seed plate by blotter method or agar plate method, while under laboratory and green house conditions resulted seedling mortality, seed rot and reducing germination indicating the transmission of *M. phaseolina* from infected seeds to seedlings (Mandhare *et al*., 2009). *Phomopsis* spp. (*Diaporthe sojae*) are associated with non germinated seeds, increase of seed infection by seed-borne *Phomopsis* spp. and other fungi seems to reduce the germination of soybean seeds (Arulnandhy, 1983). The obtained results showed that fungicidal treatments Vitavax-200 with recommended doses eliminated the fungus and improved seed germination, increased normal seedlings, decreased abnormal seedlings and seed rot of soybean cultivars under experiment conditions, earlier studies by Charjan and Tartar (1992), Singh (1997) and Sonavane *et al*. (2011). Seed treatment with 2.5 g. captan/1 kg. seed and 2.5 g. thiram/1 kg seed against *Diaporthe sojae*, *A. alternate*, *A. flavus*, *Curvularia lunata* and *F. oxysporum* improved seed germination, seedling length and dry weight (Manshi *et al*., 2004). Solanke *et al*. (1997) detected that thiram improving the germination percentage, controlled pre- and post-emergence mortality caused by *Aspergillus* spp., *F. moniliforme*, *Curvularia lunata*, *A. alternate* and *Penicillium* spp. Mean of control showed that using treatment as seed dressing of biocides (Bio-arec and Bio-zeid) reduced percentage of mean infection seed-borne fungi under study and enhanced for characters vigor of seed and seedlings soybean, these results agree with those reported by Sonavane *et al.* (2011) and Saikia *et al*. (2003). Also, Sonavane *et al*. (2011) found that *Aspergillus* spp., infection resulted in the lowest seed germination and seedling vigor index followed by *F. oxysporum*, *F. moniliforme* thin *M. phaseolina* and treatments with fungicidal (thiram+carbendazim) were significantly compared to the biological control agents (*Trichoderma viride*). Biological control of plant diseases can occur through different mechanisms, which are generally classified as: antibiosis, competition, suppression, direct parasitism, induced resistance, hypo virulence and predation (Moyer and Peres, 2008).

Present results showed that seed soaking of soybean with Salicylic acid have positive effect on reduced percentage of mean infection seed-borne fungi, number of total fungi, abnormal seedlings and seed rot besides enhancing in growth parameters such as Mean Germination Percentage (MPG), Speed Germination (SG), Seedling Length (SL) and Dry Weight (DW) compared with control, similar reasons were reported by Morsy *et al*. (2011) and Ibrahim and Kishk (2014). The mode of action of abiotic inducers for controlling plant diseases may include: (1) Acting as second messengers in enhancing the host defense mechanism (Geetha and Shetty, 2002), (2) Activating resistance by increasing the activity of peroxidase (Hassan *et al*., 2007), (3) Activating resistance through inhibition of some antioxidant enzymes and catalases (Radwan *et al*., 2008) and (4) Enhancing resistance by direct effects on multiplication, development and survival of pathogens or indirect effects on plan metabolism, with subsequent effects on the pathogen food supply (Khan *et al*., 2003). Exogenous application of SA may influence stomata closure ion uptake and transport inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan *et al*., 2003).

Generally, data obtained through this investigation showed that treatment with seed coating (Vitavax-thiram200) gave improving characters of seed and seedling vigor and eliminated or reduced numbers of pathogenic fungi, biotic and abiotic (seed dressing and soaking) inducers can be safely recommended in controlling fungal diseases and enhancing of seed and seedling vigor of soybean.

## REFERENCES


AOSA., 1983. Seed Vigor Hand Testing Book. Contribution No. 32 to the Handbook on Seed Testing. Association of Official Seed Analysis, Springfield, IL.

Agarwal, P.C., C.M. Mortensen and S.B. Mathur, 1989. Seed-borne diseases and seed health testing of rice. Technical Bulletin No. 3, Phytopathological Paper No. 30. CAB International Mycological Institute (CMI) Kew, Surrey, UK., pp: 58-59.

Anwar, S.A., S.F. Abbas, M.M. Gill, C.A. Rauf, S. Mahmood and A.R. Bhutta, 1995. Seed borne fungi of soybean and their effect on seed germination. Pak. J. Phytopathol., 7: 184-190.

Arulnandhy, V., 1983. Effect of seed-borne fungi on seed germination in soybeans. Trop. Agric., 139: 57-66.

Braccini, A.L. and O.D. Dhingra, 1996. Identification of fungi associated with soybean (*Glycine max* L. Merill) and cucumber (*Cucumis sativus* L.) seeds by different detection methods. Rev. UNIMAR., 18: 495-503.

Charjan, S. and J.L. Tarar, 1992. Effect of storage on germination and microflora of soybean (*Glycine max*) seed. Indian J. Agric. Sci., 62: 500-502.

Conca, G., F. Golinelli and A. Porta-Puglia, 1996. Health of soybean seed samples. Elelle, 42: 3-6.

Ellis, M.B., 1980. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surry, England.

Gally, T., 2006. Soybean seed diseases in Argentina. Manejo Integrado de Plagas Agroecologia, 78: 86-90.

Gally, T., B. Gonzalez, M.P. Sobero y Rojo and G. Lori, 1998. Plumele soft rot caused by *Fusarium equiseti*, *F. oxysporum* and *F. pallidoroseum* on soybean seedlings in Argentina. Plant Dis., Vol. 82. 10.1094/PDIS.1998.82.9.1063A

Geetha, H.M. and H.S. Shetty, 2002. Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide-a comparative evaluation. Crop. Prot., 21: 601-610.

Gomez, K.A. and A.A. Gomez, 1984. Statical Procedures for Agricultural Research. 2nd Edn., A Wiley Interscience Publication, New York.

Goulart, A.C.P., 2000. Health of soybean seeds produced in Mato Grosso do Sul, Brazil. J. Summa Phytopathol., 26: 346-352.

Guerrero, F.C., S.B. Mathur and P. Neergaard, 1972. Seed Health Testing of Rice: Seedborne fungi Associated with Abnormal Seedling of Rice. Vol. 37, International Seed Testing Association, Switzerland, pp: 985-997.

Haikal, N.Z., 2008. Effect of filtrates of pathogenic fungi of soybean on seed germination and seedling parameters. J. Applied Sci. Res., 4: 48-52.

Hamman, B., G. Koning and H.A. van de Venter, 1996. Germination of South African soya bean seed lots. South African J. Plant Soil, 13: 5-8.

Hassan, M.E.M., S.S. Abd El-Rahman, I.H. El-Abbasi and M.S. Mikhail, 2007. Changes in peroxidase activity due to induced resistance against faba bean chocolate spot disease. Egypt. J. Phytopathol., 35: 35-48.

ISTA., 1999. Handbook of Vigour Test Methods. 3rd Edn., International Seed Testing Association, Zurich, Switzerland, pp: 22-25.

Ibrahim, E.A.M. and A.M.S. Kishk, 2014. Enhancing viability of onion seed using some treatments. J. Plant Prot. Pathol. Mansoura Univ., 5: 179-193.

Khan, W., B. Prithivirag and D.L. Smith, 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. J. Plant Physiol., 160: 485-492.

Krishnamurty, Y.L. and K.A. Raveesha, 1996. Histopathology of soyabean seed infected with *Aspergillus flavus*. Seed Res., 24: 129-134.

Mandhare, V.K., S.B. Gawade and A.V. Suryawanshi, 2009. Detection and transmission of seed-borne infection of *Macrophomina phaseolina* causing charcoal rot in soybean. J. Plant Dis. Sci., 4: 130-131.

Manshi, G.D., R. Mandeep and R.C. Sharma, 2004. Effect of fungicidal seed treatments on *Phompsis* and other seed mycoflora of soybean. J. Res., 41: 352-355.

Medic-Pap, S., M. Milosevic and S. Jasnic, 2007. Soybean seed-borne fungi in the Vojvodina province. Phytopathol. Polonica, 45: 55-65.

Morsy, K.M., M.F. Abdel-Monaim and M.M. Mazen, 2011. Use of abiotic and biotic inducers for controlling fungal diseases and improving growth of Alfalfa. World J. Agric. Sci., 7: 566-576.

Moyer, C. and N.A. Peres, 2008. Evaluation of biofungicides for control of powdery mildew of gerbera daisy. Proc. Fla. Stat Hort. Soc., 121: 389-394.

Pioli, R.N., R. Benavidez and E.N. Marandi, 1997. Preliminary studies on pathogen incidence in fresh soyabean seeds for human consumption. Fitopatologia, 32: 116-120.

Radwan, D.E.M., G. Lu, K.A. Fayez and S.Y. Mahmoud, 2008. Protective action of salicylic acid against bean yellow mosaic virus infection in *Vicia faba* leaves. J. Plant Physiol., 165: 845-857.

Rahman, M.H., V.K. Agarwal, P.N. Thapliyal and R.A. Singh, 1995. Effect of date of sowing and seed treatment on seedling emergence and disease incidence on soybean. Bangladesh J. Plant Pathol., 11: 19-22.

Roy, K.W. and S. Ratnayake, 1997. Frequency of occurrence of *Fusarium pallidoroseum*, effects on seeds and seedlings and associations with other fungi in soybean seeds and pods. Can. J. Plant Pathol., 19: 188-192.

Saikia, R., T. Singh, R. Kumar, J. Srivastava, A.K. Srivastava, K. Singh and D.K. Arora, 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. ciceri in chickpea. Microbiol. Res., 158: 203-213.

Sinclair, J.B. and M.C. Shurtlef, 1975. Compendium of Soybean Diseases, American Psychopathological Soc., St. Paul, Minnesota, USA..

Singh, D.P., 1997. Efficacy of fungal treatment in different graded seeds in soybean. Legume Res. 20: 124-126.

Singh, D.S., A.K. Shukla and S.S. Vasuniya, 1995. Seed health studies of some advanced soybean cultivars. Indian J. Mycol. Plant Pathol., 25: 321-322.

Solanke, R.B., S.S. Kore and S.M. Sudewad, 1997. Detection of soybean seed-borne pathogens and effect of fungicides. J. Maharashtra Agric. Uniiversitie, 22: 168-170.

Sonavane, A.A., B.G. Barhate and S.J. Bade, 2011. Efficacy of bioagents and fungicides on seed mycoflora of soybean. J. Plant Dis. Sci., 6: 74-76.

Velichet, R.K. and J.B. Sinclair, 1991. Histopathology of soybean colonized by *Fusarium oxysporum*. Seed Since Technol., 19: 445-450.

Wahid, A., M.S. Javed and M. Idress, 1995. Chemicall control of *Fusarium root*, wilt and callas rot of soybean (*Glycine max* L.). Pak. J. Phytopathol., 7: 21-24.

Wilson, R.F., W.P. Novitzky and G.P. Fenner, 1995. Effect of fungal damage on seed composition and quality of soybeans. J. Am. Oil Chem. Soc., 72: 1425-1429.