Sanitary quality of soybean seeds treated with fungicides and insecticides before and after storage

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ABSTRACT – Fungi are considered as the most important among pathogens due to the higher number of species and to the damage caused both in yield and in seed quality. Thus, aimed to verify the effect of fungicide and insecticide treatment on sanitary quality and physiological performance of soybean seeds before and after storage. Seeds from cultivars NS 7494, NS 8693 and NS 7338 IPRO were used, which were analyzed separately by a completely randomized design in a 3 x 6 factorial design, being three chemical treatment applications: 1) treated and evaluated; 2) treated, stored and evaluated; 3) stored, treated and evaluated; and six mixtures of fungicides and insecticides: (Imidacloprido + Tiocarbe) + (Carbendazim + Tiram), (Imidacloprido + Tiocarbe) + (Metalaxil-M + Fludioxonil), Thiamethoxam + (Carbendazim + Tiram), Thiamethoxam + (Metalaxil-M + Fludioxonil), (Fipronil + Piraclostrobina + Metil-tiofanato) and the control, which was added only water. The healthy test, germination, cold and accelerated aging tests were evaluated. Seed treatment products require at least two months to be effective in controlling Penicillium spp. and Fusarium spp. The mixtures containing Carbendazim + Tiram in its composition are efficient in the control of pathogens regardless of the application time of products.

Index terms: Fungi, Fusarium spp., Penicillium spp., Aspergillus spp.

Introduction

In order to achieve high soybean yields, it is necessary to use high quality seeds associated with management practices such as seed treatment. Scheeren et al. (2010) found that the yield resulting from seed lots with high vigor may be 9%
higher than with low vigor. Such vigor is resulting from the sum of the processes performed throughout the production and postharvest.

In the production of soybean seeds, the harvest does not coincide with the ideal time for the planting of the subsequent harvest, which requires a long storage period of the seeds until sowing. Thus, their quality should be maintained until the ideal moment to take them to the field (Galli et al., 2007). In this way, storage is a step in which the rate and intensity of the deterioration process should be reduced in order to maintain the initial quality of the lots. Therefore, the storability of seeds is influenced both by the initial quality of the lots as well as by storage conditions (Catão et al., 2010). Nevertheless, ideal storage conditions may favor the survival of several storage pathogens, including fungi from the genus Aspergillus spp., Penicillium spp. and Fusarium spp.

The storage fungi are present in the newly harvested seeds, usually at low percentages and are capable to survive in low moisture environment, proliferating in succession to field fungi and causing seed deterioration (Rocha et al., 2014). The pathogen attack on soybean seeds is one of the factors that leads to the physiological quality loss of seeds, reducing the germination and the vigor of the lots, which end up precluding the final stand of the crop, resulting in productivity and economic losses to the farmer. Fungi are considered as the most important among pathogens due to the higher number of species and to the damage caused both in yield and in seed quality.

In this context, it is necessary to use seed treatment, which is a technology that, when associated with plant genetic engineering and biotechnology, allows high soybean yield and producer satisfaction in meeting market demands (Ferreira et al., 2016). This process consists of applying compounds capable of protecting seeds against deleterious effects caused by pathogens, aiding to control storage fungi and protecting the seedlings in the initial establishment period of the crop, favoring sowing, emergence and development (Balardin et al., 2011).

Currently, seed treatment is performed industrially, reducing risks to the operators and the environment. However, certain limitations are worrying, including the possible effects of active ingredients on seed quality during storage and in the field (Brzezinski et al., 2015). Dan et al. (2013) found reductions in the emergence of seedlings derived from soybean seeds treated with thiamethoxam insecticide during storage. Vanin et al. (2011) found that the treatment of sorghum seeds with the acephalate active ingredient reduced the germination rate and emergence of seedlings due to phytotoxicity.

Thereby, there is a need for studies evaluating the effect of the most commonly used seed treatment products and the correct application time on the sanitary quality and the physiological performance of soybean seeds.

Thus, the aim of the present study was to verify the effect of fungicide and insecticide treatment on sanitary quality and physiological performance of soybean seeds before and after storage.

**Material and Methods**

The experiment was performed at the Universidade Federal de Lavras, Lavras, MG, Brazil, in the Departments of Agriculture and Plant Pathology, with physiological analyses performed at the Central Laboratory of Seed Analyses, and phytosanitary analyses at the Laboratory of Seed Pathology.

The seeds used to perform the experiment were supplied by the company Nidera Sementes. Seeds from cultivars NS 7494, NS 8693 and NS 7338 IPRO were used, which were analyzed separately by a completely randomized design in a 3 x 6 factorial design, being three chemical treatment applications and six mixtures of fungicides and insecticides.

Each cultivar was subjected to three treatments, which differed with one another by the time they received the chemical treatment: 1) treated and evaluated (seeds were evaluated immediately after chemical treatment); 2) treated, stored and evaluated (the seeds were stored with chemical treatment for two months and then evaluated); 3) stored, treated and evaluated (the seeds were stored without chemical treatment for two months, and then were treated and evaluated immediately).

In order to accomplish the treatment of seeds, the products were combined as follows with the respective dosages: T1 - (Imidacloprido + Tiocarbe) - (5 mL.Kg⁻¹) + (Carbendazim + Tiram) - (2 mL.Kg⁻¹); T2 - (Imidacloprido + Tiocarbe) - (5 mL.Kg⁻¹) + (Metalaxil-M + Fludioxonil) - (1 mL.Kg⁻¹); T3 - Thiamethoxam - (2,5 mL.Kg⁻¹) + (Carbendazim + Tiram) - (2 mL.Kg⁻¹); T4 - Thiamethoxam - (2,5 mL.Kg⁻¹) + (Metalaxil-M + Fludioxonil) - (1 mL.Kg⁻¹), T5 - (Fipronil + Piraclostrobina + Metil-tiofanato) - (2 mL.Kg⁻¹) and the control (T6), which was added only water.

Plastic bags with 2 kg capacity were used for the application of products in the seeds. The products were previously mixed in Petri dish (fungicide + insecticide + water) and placed in the plastic bags, being added finally 500 g of seeds. The whole was stirred until obtaining a homogeneous mixture of seeds. After the treatment, the seeds were arranged in the shade at a temperature of approximately 25 °C for 20 min in order to dry the product on the seed surface. The treated seeds were conditioned in corrugated fiberboard packages and kept for two months in a conventional warehouse under uncontrolled conditions, with an average temperature of 20.4 °C and
relative humidity of 65.5% (Dantas et al., 2007).

For the healthy test, the incubation method was used on filter paper without freezing (Neergaard, 1977), with eight subsamples of 25 seeds. The seeds were distributed in 15 cm diameter Petri dishes containing three filter paper sheets previously sterilized and moistened with sterilized water, agar and 2,4 D. The plates were incubated at 20 °C and 12 h photoperiod, where they remained for seven days, being then evaluated in relation to the presence of pathogens (Brasil, 2009). A stereoscopic magnifying glass and an optical microscope were used to identify the pathogens present in the seeds. The incidence was evaluated as percentage of found fungi.

Germination, cold and accelerated aging tests were performed in order to verify the physiological performance of seeds by sanitary quality.

In the germination test, four replicates of 50 seeds per plot were used, seeded in two germination sheets of paper and covered by one sheet, moistened with distilled water in an amount equivalent to 2.5 times the weight of the dry paper. The seedlings were kept in a germinator at 25 °C and the evaluations were conducted at five (first germination count) and at eight days after sowing, being the results expressed as percentage of normal seedlings (Brasil, 2009).

In the cold test, four subsamples of 50 seeds were used. The sowing was performed in 2:1 soil + sand substrate, contained in plastic trays and moistened with 70% retention capacity. After sowing, the trays were placed in a cold room at 10 oC for seven days and then transported to the plant grow room at 25 °C for another seven days, thus obtaining the number of emerged seedlings. Results were expressed as a seedling percentage.

In accelerated aging test, samples with 250 seeds were placed in a gerbox plastic boxes with 40 mL of distilled water. These boxes were incubated at 42 °C for 48 h in a BOD chamber (Marcos-Filho, 1999). Then, the germination test was performed with four subsamples of 50 seeds. The evaluation occurred five days after sowing. Results were expressed as average percentage of normal seedlings (Brasil, 2009).

Statistical analysis was performed using statistics Sisvar statistical software (Ferreira, 2014). In analyses, when a significant effect of treatments was verified, averages of Scott-Knott test at 5% of probability were used to test the significance of differences among treatment averages. The values of the fungal incidence were previously transformed into (√x+1).

Results and Discussion

The germination test was realized for treatments characterization (Table 1). Regardless of the product used for the treatment and of cultivars, seed germination was reduced in the treated and stored and the smallest seedlings were obtained when the seeds quality after storage were assessed. This reduction in germination of seeds is due to many circumstances such as the environmental conditions during the production of seeds, insect attack, lipid and water content in seeds, presence of mechanical damage arising from transport and processing, storage conditions, and especially, in the case of seed with high quality, of the fungi attack and chemicals used in seed treatment (Sales et al., 2011). In this study, when the seeds were stored there was phytotoxic effect treated by the contact time of the products with the seeds. And when the treatment was done after there was storage phytotoxicity by the entry of products within seeds due to the damage caused by storage fungi.

There was a significant effect on the three cultivars of the storage fungi Fusarium, Penicillium and Aspergillus.

Table 1. Germination (%) of seeds from three soybean cultivars treated with different products at different time of application.

| Cultivars       | Application Time | Products Treat | Treat + Stored | Stored + Treat |
|-----------------|------------------|----------------|---------------|---------------|
| T1              | 87 aC            | 83 bC          | 70 cD         |               |
| T2              | 89 aC            | 92 aB          | 71 bD         |               |
| T3              | 97 aA            | 91 bB          | 98 aA         |               |
| T4              | 94 aB            | 85 bC          | 81 cC         |               |
| T5              | 93 bB            | 97 aA          | 80 cC         |               |
| T6              | 95 aB            | 85 cC          | 91 bB         |               |
| NS 7494 (CV=2.26%) | T1              | 90 aB          | 92 aA         | 57 cC         |
|                 | T2              | 91 aB          | 91 aA         | 56 bC         |
|                 | T3              | 95 aA          | 87 bB         | 82 cA         |
|                 | T4              | 81 bB          | 92 aA         | 79 bA         |
|                 | T5              | 87 aB          | 85 aB         | 73 bB         |
|                 | T6              | 95 aA          | 94 aA         | 70 bB         |
| NS 8693 (CV=3.50%) | T1              | 87 aB          | 74 bD         | 64 cD         |
|                 | T2              | 91 aB          | 88 aB         | 73 bC         |
|                 | T3              | 96 aA          | 85 bC         | 84 bB         |
|                 | T4              | 93 aA          | 90 aB         | 90 aA         |
|                 | T5              | 92 aA          | 85 bC         | 86 bB         |
|                 | T6              | 90 bB          | 98 aA         | 86 bB         |

Averages followed by the same lowercase letter on the rows and capital on the columns did not differ among themselves by Scott-Knott test at 5% probability. T1 - (Imidacloprido + Tiofcarbe) + (Carbendazim + Tiram); T2 - (Imidacloprido + Tiofcarbe) + (Metalaxil-M + Fludioxonil); T3 - Thiamethoxam + (Carbendazim + Tiram); T4 - Thiamethoxam + (Metalaxil-M + Fludioxonil); T5 - (Fipronil + Piriacrostobina + Metil-tiofanato); T6 – control.
**Fusarium**

The mixtures containing Carbendazim + Tiram in its composition controlled more efficiently the fungus regardless of the treatment time for the NS 7494 cultivar (Figure 1A).

Storage by itself contributed to reduce the percentage of *Fusarium* in seeds from the NS 7494 cultivar, except when Imidacloprido + tiocarbe + Metalaxil-M + fludioxonil and Fipronil + piraclostrobina + metil-tiofanato was used after two months of storage, thus obtaining a higher incidence of *Fusarium* in relation to control. These products also act to reduce this fungus when in contact with the seeds for a longer period (at least two months). The same occurred in seeds from the NS 8693 cultivar, which were treated and stored with the mixtures based on Metalaxil-M + fludioxonil (Figure 1B).

In the NS 8693 cultivar, all treatment products were efficient in reducing *Fusarium* in relation to control.

For the NS 7338 IPRO cultivar, the treatment in contact with the seeds for two months contributed to eliminate the *Fusarium* regardless of the product used as treatment (Figure 1C).

In the NS 7338 IPRO cultivar, the treatments Imidacloprido + tiocarbe + Metalaxil-M + fludioxonil, Thiamethoxam + Metalaxil-M + fludioxonil and Fipronil + piraclostrobina + metil-tiofanato were not efficient to reduce *Fusarium* in newly harvested seeds that did not undergo any type of storage. However, when seeds were stored and treated before or after storage for two months, these products reduced the incidence of the fungus.

Despite the efficiency of the treatment and storage products in the control of the fungus, the germination and vigor was reduced in most of infested seeds, which are those that did not undergo any type of storage and treatment, as can be observed in the cold test (Juhász et al., 2013).

The cold test is important both to select the lots for sowing and to test the fungicide efficiency in relation to the persistence of the product and the phytotoxicity in the seedlings, since by being in direct contact with the surface of seeds in a high moisture condition, the product can be absorbed by the seed or leached.

For seeds from the NS 7494 cultivar treated before the quality tests, there was a better performance in the vigor of seeds that received the Imidacloprido + tiocarbe + Carbendazim + Tiram treatment and was maintained with the Fipronil + piraclostrobina + metil-tiofanato treatment by the cold test (Table 2). The remainder of the products contributed to a reduced vigor. Based on the principle of the cold test, it can be verified that when the seeds were sown and left for seven days at a temperature of 10 °C under humidity of 70% of the retention capacity, there was abrupt entry of treatment products into the seeds, causing a below-expected performance for the referred cultivar, probably due to phytotoxicity. Thus, the product was not efficient, since there was no *Fusarium* control as can be observed in Figure 1.
Penicillium

There was a reduced incidence of *Penicillium* when the seeds from the cultivar NS 7494 were treated with Imidacloprido + Tiocarbe + Carbendazim + Tiram and with Thiamethoxam + Metalaxil-M + fludioxonil regardless of the time when the treatment was performed (Figure 2A).

All treatment products acted to reduce the *Penicillium* when the treatment was performed immediately after the harvest in relation to the control. However, the combination that most contributed to the reduction had the products Imidacloprido + Tiocarbe + Carbendazim + Tiram, which reduced the incidence of fungus in the control from 54% to 0% (Figure 2A).

The treatment Thiamethoxam + Metalaxil-M + fludioxonil was efficient for controlling *Penicillium* only when the seed was stored already treated, which offered a protection to the seeds, since the storage fungi such as *Penicillium* tends to increase after storage. In contrast, the Imidacloprido + Tiocarbe + Metalaxil-M + fludioxonil did
not differ from the control in relation to the percentage of fungus incidence (Pereira et al., 2007).

In the cultivar NS 8693, when treatment with Imidacloprido + tiocarbe + Metalaxil-M + fludioxonil was performed after two months of storage and after harvesting, there was a higher incidence of fungus, which allows observing that this product is more efficient when it stays in contact with the seeds for at least two months (Figure 2B). For this same cultivar, the treatments containing Carbendazim + Tiram in its composition were efficient in the control of *Penicillium*, regardless of the treatment time of the seeds. The Carbendazim + Tiram fungicide contains in its composition the active carbendazim, from the benzimidazole group, and Tiram, from the dimethylthiocarbamate group. According to Goulart et al. (2015), the fungicides from the benzimidazole group act to inhibit the DNA synthesis of fungi, whereas those from the dimethylthiocarbamate group act to inactivate the essential enzymes. Therefore, this fungicide is extremely efficient in fungi control and seed protection.

For both the cultivars NS 8693 and the NS 7338 IPRO, the storage favored the reduction of the incidence of *Penicillium* in seeds treated with Thiamethoxam + Metalaxil-M + fludioxonil and Fipronil + piraclostrobina + metil-tiofanato, and the control (Figures 2B and 2C). For these two cultivars, all treatment products were efficient in reducing *Penicillium* regardless of the time when seed treatment was performed, except when using the product Fipronil + piraclostrobina + metil-tiofanato in the treatment of freshly harvested seeds from the cultivar NS 8693 and the product Thiamethoxam + Metalaxil-M + fludioxonil in NS 7338 IPRO cultivar, which did not differ from the control.

Furthermore, in the accelerated aging test (Table 3), in which high moisture and high temperatures favor fungal proliferation, it was verified that it showed high vigor for treated and stored seeds and low vigor for the seeds treated before the quality tests in any one of the cultivars, which confirms that the contact time of fungicides with the seeds is relevant for the complete elimination of the fungi before planting, since pathogens present in the soil or in the seeds reduce the stand of soybean plants (Costamilan et al., 2012). Besides the fungicide treatment does not reduce the physiological quality of seeds, it is extremely efficient in the control of pathogens such as *Penicillium*, controlling 100% of the pathogen population (Figure 2).

For any of the three cultivars, the Imidacloprido + tiocarbe + Carbendazim + Tiram mixture was efficient in reducing *Penicillium* regardless of the treatment time.

### Aspergillus

There was an increase of *Aspergillus* along the seed storage of the cultivars NS 7494 and NS 8693 that served as controls and those that were treated with Imidacloprido + tiocarbe + Metalaxil-M + fludioxonil (Figures 3A and 3B). However, the combination of treatment Thiamethoxam + Metalaxil-M + fludioxonil in contact for two months with the seeds from cultivars NS 7494 and NS 7338 IPRO was efficient in reducing the fungus (Figures 3A and 3C).

Seeds treated with Imidacloprido + tiocarbe + Metalaxil-M + fludioxonil and stored did not differ from untreated seeds, showing that this treatment was not efficient in the control of the fungus, for both cultivars NS 8693 and NS 7338 IPRO (Figures 3B and 3C). Additionally, when the seeds were treated soon after harvest, this product did not act to reduce the fungus for the NS 7338 IPRO cultivar, since there was no difference in the control (Figure 3C).

### Table 3. Accelerated aging from three soybean cultivars according to the applied products and the application time.

| Cultivars     | Products | Application time |
|---------------|----------|-----------------|
|               |          | Treat | Treat + Stored | Stored + Treat |
| NS 7494       |          |       |                |                |
| (CV=2.87%)    | T1       | 42 aA | 85 aA          | 78 bA          |
|               | T2       | 26 cB | 76 aD          | 71 bB          |
|               | T3       | 42 cA | 86 aA          | 80 bA          |
|               | T4       | 24 cB | 76 aD          | 63 bC          |
|               | T5       | 21 cC | 82 aB          | 70 bB          |
|               | T6       | 21 cC | 80 bC          | 71 bB          |
| NS 8693       |          |       |                |                |
| (CV=3.72%)    | T1       | 22 cA | 82 aA          | 63 bA          |
|               | T2       | 17 cB | 80 aB          | 63 bA          |
|               | T3       | 22 cA | 70 aC          | 57 bB          |
|               | T4       | 3 cD  | 65 aD          | 52 bC          |
|               | T5       | 14 cC | 62 aE          | 53 bC          |
|               | T6       | 6 cD  | 84 aA          | 48 bD          |
| NS 7338       |          |       |                |                |
| IPRO          | T1       | 69 bA | 73 aB          | 59 cC          |
| (CV=4.36%)    | T2       | 48 cB | 72 aB          | 62 bC          |
|               | T3       | 34 cD | 73 aB          | 69 bB          |
|               | T4       | 43 cC | 63 aC          | 54 bD          |
|               | T5       | 11 bE | 83 aA          | 86 aA          |
|               | T6       | 7 cF  | 25 bD          | 61 aC          |

Averages followed by the same lowercase letter on the rows and capital on the columns did not differ among themselves by Scott-Knott test at 5% probability. T1 - (Imidacloprido + Tiocarbe) + (Carbendazim + Tiram); T2 - (Imidacloprido + Tiocarbe) + (Metalaxil-M + Fludioxonil); T3 - Thiamethoxam + (Carbendazim + Tiram); T4 - Thiamethoxam + (Metalaxil-M + Fludioxonil); T5 - (Fipronil + Piraclostrobina + Metil-tiofanato); T6 – control.
For the three cultivars, the products Imidacloprido + tiocarbe + Carbendazim + Tiram, Thiamethoxam + Carbendazim + Tiram and Fipronil + piraclostrobina + metiltiofanato were efficient to reduce the *Aspergillus*, regardless of the application time of products.

Conclusions

The seed treatment before the quality tests was not detrimental to seed quality under optimum conditions; however, under stress conditions, the seed performance was compromised due to the product concentration, except for the use of mixtures containing Thiamethoxam insecticide in the composition.

The contact time of products with the seeds was not sufficient to reduce the incidence of the fungi *Penicillium*, *Aspergillus* and *Fusarium*, except by using products based on Carbendazim + Tiram, which contributed to the reduction of all fungi, regardless whether the seeds were stored or not.

When using the products Imidacloprido + tiocarbe + Carbendazim + Tiram, there was better expression of the physiological quality of treated and stored seeds.

Seed treatment products require at least two months to be effective in controlling *Penicillium* spp. and *Fusarium* spp.

The mixtures containing Carbendazim + Tiram in its composition are efficient in the control of pathogens regardless of the application time of products.

The storage acts in order to reduce the *Fusarium* spp. and increases the *Aspergillus* spp.

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Figure 3. Incidence (%) of *Aspergillus* spp. in seeds from three soybean cultivars according to the treatment of seeds products and the application time. Averages followed by the same lowercase letter on the application time and capital on the treatment of seeds products did not differ among themselves by Scott-Knott test at 5% probability. The original averages were presented, but the data were compared as a function of the transformed data (Transformation to $(x+1)^{0.5}$).
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