Wheat Seed Quality Assessment After Thiamethoxam Treatment: Integrating Optical Techniques with Standard Methods

Avaliação da qualidade de sementes de trigo após tratamento com Tiametoxam: Integração de técnicas ópticas com métodos padrão

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ABSTRACT

Insecticide treatment of seeds protects against early field pests. Therefore, studies on the physiological and biochemical responses of crops exposed to insecticides are required. Standard (non-optical) tests are used to evaluate the physiological quality of seeds. However, some tests may need to be more sensitive to observe possible differences between the control group and the groups of interest. Optical techniques such as ultra-weak photon emission and Fourier transform infrared spectroscopy have been discovered to aid standard tests. The effects of different doses of Thiamethoxam were evaluated on wheat seeds from the cultivar FPS Certero. Samples were divided into five groups: control and treated with 75, 150, 225, and 300 mL of Thiamethoxam for each 100 kg of seeds. The ultra-weak photon emission results showed that it was possible to classify the treatment doses through the intensity of photon emissions. The Fourier transform infrared spectroscopy analyses can be used for biological analysis but are unrelated to Thiamethoxam seed treatment. Considering the findings, optical techniques can be used as an additional tool for assessing the physiological quality of seeds.

Keywords: Insecticide Treatment; Physiological Responses; Thiamethoxam; Ultra-weak photon emissions; Fourier transform infrared spectroscopy;
RESUMO

O tratamento com inseticida protege as sementes contra as pragas iniciais do campo, necessitando de estudos sobre as respostas fisiológicas e bioquímicas. São utilizados testes padrão (não ópticos), mas alguns testes podem necessitar de maior sensibilidade para observar possíveis diferenças entre os grupos de controle e de interesse. Técnicas ópticas como a emissão de fótons ultra-fraca e a espectroscopia de infravermelho com transformada de Fourier podem auxiliar. Desta forma, foram avaliados os efeitos de diferentes doses de tratamento com o Tiamethoxam em sementes de trigo do cultivar FPS Certero. As amostras de sementes foram divididas em cinco grupos: controle e as tratadas com 75, 150, 225 e 300 mL de Tiamethoxam para cada 100 kg de sementes. A emissão de fótons ultra-fraca mostrou que era possível classificar as doses de tratamento através da intensidade das emissões de fótons. As análises de espectroscopia de infravermelho com transformada de Fourier, não apresentaram resultados que pudessem ser associados ao tratamento. Considerando os resultados encontrados, as técnicas ópticas apresentam potencial de serem utilizadas como ferramentas adicionais para a avaliação da qualidade fisiológica das sementes.

Palavras-chave: Tratamento com Inseticida; Respostas Fisiológicas; Tiamethoxam; Emissões de Fótons ultrafraca; Espectroscopia de Infravermelho com Transformada de Fourier;

INTRODUÇÃO

Wheat is one of the three most essential cereals grown worldwide because of its nutritional and economic aspects (COSTA; SOUZA; STAMFORD, 2008; DEMIR; ONDE; SEVERCAN, 2014). Due to the great importance of wheat as a food crop, guaranteeing seed quality is extremely important. In this sense, seed quality is defined as a set of inherent characteristics that indicate the seed's performance potential. The attributes that define seed quality are genetic, physical, physiological, and sanitary (CONAB, 2017; MARCOS FILHO, 2005). With the need to establish plant populations quickly and uniformly, interest in identifying the physiological quality of crop seeds has increased. As a result, of the four attributes that determine seed quality, the physiological component is the most significant interest, as it gathers information on seed viability (germination) and vigor (MARCOS FILHO, 2005; MARCOS FILHO, 2017). Genetic factors, the occurrence of pathogens, mechanical damage caused to seeds, the occurrence of insects and harmful microorganisms, and chemical treatments with fungicides, bactericides, and insecticides can affect the physiological quality of seeds (CARVALHO; NAKAGAWA, 2000; MARCOS FILHO, 2005; MARCOS FILHO, 2013).

Seeds are often treated with agrochemicals to increase the initial performance of crops, providing seeds and seedlings protection against pests and insects (CASTRO et al., 2008; MACEDO; CASTRO, 2011). However, some agrochemicals can also affect their physiological quality, as with hormone control agrochemicals (MACEDO; CASTRO, 2011). Within this group of agrochemicals, there is the subgroup classified as
bioactivators, which includes the systemic insecticide of the neonicotinoid chemical group containing the active ingredient Thiamethoxam (THX) (CASTRO, 2006). Some articles describe those pesticides containing the THX have shown effects on the physiological quality of seeds, such as increased vigor, increased photosynthetic rate, phytomass synthesis, more profound root formation, and the consequent better use of the crop's productive potential (ALMEIDA et al., 2012; CASTRO et al., 2008). However, the effects of THX on the physiological quality of seeds during the plant's life cycle still need to be fully understood, and their potential use in agriculture needs to be verified (MACEDO; CASTRO, 2011; MACEDO, 2012).

The Seed Analysis Rules (RAS) describe methods for assessing the physiological quality of seeds, such as the germination test (physiological) and tests based on seedling performance or characteristics (BRASIL, 2009; MARCOS FILHO, 2005). However, these tests can be time-consuming and labor-intensive and produce more inconsistent results (MARCOS FILHO, 2005; WIJEWARDANA; REDDY; BELLALOUI, 2018). In this context, the optical techniques of Ultra-Weak Photon Emission (UWPE) and Fourier Transform Infrared Spectroscopy (FTIR), operating in the Attenuated Total Reflectance (ATR) mode, have the potential to assess the physiological quality of seeds as fast and low-cost (FAN; MA; WU, 2020). The UWPE technique makes it possible to analyze vigor (YU; WANG, 2010; WANG; YU, 2009). FTIR-ATR, on the other hand, provides information on organic compounds (CALABRÒ; MAGAZÙ, 2016), making it possible to differentiate between species of the same seed and can be applied in the process of assessing seed quality (SUCHOWILSKA et al., 2012).

UWPE refers to the highly low-level light emissions biological systems generate. As a result, biochemical reactions are typically associated with oxidative metabolic processes and are detectable using susceptible photomultiplier tubes or charge-coupled device (CCD) cameras (CIFRA; POSPÍŠIL, 2014; MOULD et al., 2024). Some studies have found that plants respond to fungi, hormones, temperature changes, and herbicides (KATO et al., 2014). Gallep and Santos (2007) studied the germination of wheat seeds in wastewater sediment solutions which it was observed that the intensity of UWPE, as well as its increase over some time, is related to germination, and the time profiles are altered with the different concentrations (GALLEP; SANTOS, 2007). The authors Wang and Yu (2009 and 2010) studied the relationship between the intensity of UWPE and the level of vigor of seeds irradiated with different doses of gamma radiation and the intensity of UWPE of the flour of these seeds with the dose of radiation applied where they concluded
that the analysis of the intensity of UWPE can be used to distinguish the difference in the level of vigor of wheat and rice seeds (YU; WANG, 2010; WANG; YU, 2009). Moraes et al. (2010) carried out a study involving spontaneous light emission with wheat seedlings treated with potassium dichromate (K₂Cr₂O₇), in which they observed that the chemical stressor was neutralized by the plants’ metabolism up to a specific concentration limit, after which it begins to have a deleterious effect on germ development, causing a reduction in root length and total germination rate and as the concentration of K₂Cr₂O₇ increased, photocounts decreased over time when compared to the control group (MORAES et al., 2010). The work by Bertogna et al. (2013) analyzed the patterns of UWPEs in wheat germination tests under chemical stress by mercury (Hg) and sodium fluoride (NaF) solutions, observing that the seeds irrigated with the chemical solutions showed lower development and higher photocounts compared to the seeds used in the control tests (BERTOGNA et al., 2013).

For some time, the FTIR technique was used exclusively to analyze materials in the chemical industry, but nowadays, the method has also been applied to biological and biomedical analysis (ALAWAN, 2014). One of the examples found in the literature is the work by Amir et al. (2011), in which FTIR was used to identify the chemical composition of different varieties of wheat seeds (AMIR et al., 2011). In 2015, Demir, Onde, and Server applied FTIR - ATR to show the phylogenetic relationship between Triticum and Aegilops wheat species. This work found the method to be sensitive, fast, and low-cost, capable of discriminating wheat seeds at genus and species levels (DEMIR; ONDE; SERVERCAN, 2015). Larios et al. (2020) presented the FTIR - ATR technique associated with chemometric methods to distinguish the level of vigor of soybean seeds (Glycine max L.) with their dimensionality reduced through multivariate analysis using the Principal Component Analysis (PCA) technique, which were analyzed with some learning algorithms and, with this, it was possible to differentiate the seed lots (LARIOS et al., 2000b).

Therefore, this article combines alternative and complementary techniques with traditional techniques. The proposed technique compares the temporal profile of UWPEs, their molecular components, and physiological parameters to untreated seeds (control group) to evaluate the physiological quality of wheat seeds treated with varying doses of the commercial THX product.
MATERIALS AND METHODS

Figure 1 presents a flowchart with the materials and methods used to obtain data for analysis.

**Figure 1 - Materials and Methods**

**TREATMENT OF THE WHEAT SEED SAMPLES**

The Cruiser® 350 FS commercial product (Syngenta Proteção de Cultivos Ltda., São Paulo - SP, lot number 0070-17-11860) was used for the seed treatment. It contains 350 g of THX active ingredient per liter (SYNGENTA, 2021). Wheat seeds of *Triticum aestivum* L., cultivar FPS Certero, lot number 037, 2018 crop, grown in Arapoti - Paraná State/Brasil (-24.26, -50.11) were used. The sample was divided into five groups: 0 (control group), 75, 150, 225, and 300 mL of THX for each 100 kg of seeds.

**UWPE DATA ACQUISITION**

The temporal photon count profiles were obtained using custom-built equipment. An aluminum darkroom houses a photomultiplier (PMT) photon counting module (H6240-01 model, from Hamamatsu Corp.). The instrumentation includes a dark chamber for the Petri’s dish where the samples are placed, an electronic acquisition board for acquiring the counting pulses from the PMT module, thermal stabilizations for both the photon counting module and the seed samples, and a virtual instrument program running on a personal computer for acquiring and displaying the photon counting data; more information can be found in (BERTOGNA et al., 2016). A temperature controller kept the interior of the dark chamber, where the Petri dish was found, at 22.0 °C. The photon count data were smoothed by the local average of one hundred adjacent counts to reduce noise variations and then transferred to the computer. The counts were integrated in 10
seconds (/10 s). First, the dark noise of the darkroom was measured through the average of three samples, and the result was 158.0 ± 0.6 photons / 10s. The analyses were conducted in triplicate (asynchronous), with forty wheat seeds for each test. The seeds were placed in a Petri dish (8 cm in diameter) with five sheets of qualitative filter paper inside (both previously sterilized). Then, 6 mL of deionized water (Cloroquímica®, pH 7) was applied to the seeds. The sample was left in a germination room at 22.0 °C for 72 h. So, the sample received an added 2 ml of the same water. Afterward, the samples were transferred to the photon counting chamber under low light conditions to collect UWPE data for 48 hours. After the tests had been completed, the averages of temporal slope profiles were calculated for each group, and the total photon counts for each group were computed. In addition, the Scott-Knott test (five percent probability) and the Dunnet test (five percent probability) were performed.

**MEASUREMENTS OF THE LENGTHS OF THE AERIAL PART AND THE PRIMARY ROOT OF NORMAL SEEDLINGS**

The physiological parameters measured were lengths of the aerial part (LA) and the primary root (LR) of normal wheat seedlings (classified according to Brasil (2009). The research was conducted at the Federal University of Technology - Paraná State - Brazil. Five hundred seeds were randomly selected for the five groups listed in the treatment of the wheat seed samples. These seeds were initially split into five groups of 100 each; the first division corresponded to the five days the analysis was conducted. After the initial division, the 100 seeds were split into four groups with 25 seeds. This division refers to the number of samples from the same group analyzed on the same day. Thus, 100 rolls with 25 seeds each were prepared. Every 24 hours, four rolls from each sample were removed and analyzed, never to be returned to the germination chamber. The test was developed as follows: seed germination papers (J. Prolab®) were weighed and moistened with a volume of deionized water equal to two and a half times the dry substrate's mass. The 25 seeds were then placed on two paper sheets and covered with a third one. Following that, rolls were made and placed in plastic bags, and the rolls were then placed in a 75° tilted stand in a photoperiodic germination chamber (Solab®, SL 224 model), previously disinfected with 70% alcohol and regulated at a constant temperature of 20.0°C under a 12-hour photoperiod. The procedures’ steps were adapted from Brasil (2009) and Nakagawa (1999). Following the conclusion of the testing, the Scott-Knott test (five percent probability) and the Dunnet test (five percent probability) were performed.
SAND EMERGENCE MEASUREMENTS

The experiment used an entirely randomized design. Plastic trays were filled with 2 kg of autoclave-sterilized sand, and 300 mL of water was spread over the sand. Then, at a depth of about 0.05 m, a group of one hundred seeds was sown in the sand. They were kept in the germination room for eight days at a constant temperature of 20.0 °C and under continuous lighting. Four repetitions were performed for each group. The procedures' steps were established by the Rules of Seed Analysis (BRASIL, 2009). The following parameters were examined: the first count of emergence (FCE - performed on the fourth day after the start of the test), the last count of emergence (LCE - performed on the eighth day after the beginning of the test where the number of normal seedlings was recorded). In addition, the speed of emergence index (SEI) was calculated using the equation from Maguire (1962). When the examinations were over, the Scott-Knott test (five percent probability) and the Dunnet test (five percent probability) were performed.

FTIR DATA ACQUISITION

Every 24 hours, a set of quadruplicates for each group were removed from the germination chamber to realize normal seedling aerial part and primary root length measurements, as detailed in the section measurements of the lengths of the aerial part and the primary root of normal seedlings. Only three random samples were applied in this step. The normal seedlings were dried in a thermoelectric oven (Sterilifer®) at 80.0 °C for 24 hours. Furthermore, the external and internal pericarp and teguments of the seeds were dried separately in the oven. The LA and LR measurements were not performed on the first and second days after the test installation (no significant growth occurred), but these samples were dried. The seedlings, external and internal pericarp, and teguments of the seeds were mixed and macerated. Then, the samples were placed in individual glass vials previously sterilized and stored in a desiccator until the FTIR tests began. A spectrometer (Varian®, 640 IR model) was used in those experiments. In 32 scans, a universal attenuated total reflectance (ATR) sampling accessory (Pike®, Miracle model) with a Zinc Selenide crystal with a resolution of 4 cm⁻¹ and a range of 4000 to 650 cm⁻¹ was used. The fingerprint region (800 to 1500 cm⁻¹) was selected to analyze each spectrum. Each day, three spectra for each group were analyzed. They were normalized by a wavenumber centered at 997 cm⁻¹. The dimensionality of the spectra was then reduced using the chemometric method: the principal component analysis (PCA) technique.
RESULTS AND DISCUSSIONS

UWPE DATA RESULTS

Figure 2 shows the three temporal slope profile averages associated with the 48-hour UWPE data acquired from the five wheat seed groups. The profiles revealed similarities between the groups, with photon counts increasing over time. This behavior could be related to the seedlings' increased metabolism. However, the photon counts are lower in the stressed groups (treated with 225 and 300 mL of THX). As Pospíšil (2014) mentioned, the total germination rate is directly associated with the intensity of emissions, and the hormones used to stimulate and inhibit seed development alter the temporal profile and average intensity of UWPEs.

Figure 2 - Temporal Slope Profiles Average

![Temporal Slope Profiles Average](image)

Source: Authors (2024)

Table 1. Temporal slope profiles and Total photon counts Average

<table>
<thead>
<tr>
<th>Dose of THX (mL)</th>
<th>Temporal slope profiles Average (x 10^-4) (arbitrary units)</th>
<th>Total photon counts average (x 10^6) (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.52 ± 0.45, a</td>
<td>3.65 ± 0.04, a #</td>
</tr>
<tr>
<td>75</td>
<td>1.99 ± 0.75, a</td>
<td>3.59 ± 0.02, a</td>
</tr>
<tr>
<td>150</td>
<td>2.10 ± 0.55, a</td>
<td>3.50 ± 0.05, b *,</td>
</tr>
<tr>
<td>225</td>
<td>0.92 ± 0.17, b</td>
<td>3.12 ± 0.03, c *, #</td>
</tr>
<tr>
<td>300</td>
<td>1.04 ± 0.27, b</td>
<td>3.04 ± 0.03, d *, #</td>
</tr>
<tr>
<td>Average</td>
<td>1.51</td>
<td>3.38</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>32.01</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Note: In the column, the average followed by the same lower-case letter does not differ statistically by Scott-Knott test, 5% probability; the average followed by * differs statistically from the control group, by Dunnett test, 5% probability; the average followed by # differ statistically from the group treated with 150 mL of THX, by Dunnett test, 5% probability.

Source: Authors (2024)

Scott-Knott’s test revealed a significant difference between the average temporal slope profiles of the group treated with the recommended dose (150 mL of THX), the control group, and the lowest treatment dose from the stressed groups (225 and 300 mL of THX) indicated in the letters in Table 1. The Scott-Knott test revealed a significant
difference between the average total photon counts of the group treated with the recommended dose (150 mL of THX) and stressed groups (225 and 300 mL of THX) indicated in the letters in Table 1. Furthermore, the association between the control group and the group treated with the lowest dose significantly differs from that of other groups. Dunnett’s test revealed that the group treated with the recommended dose and the most stressed groups significantly differed from the control group, as shown by the symbol * in Table 1. Furthermore, the most stressed groups vary substantially from those treated with the recommended dose, as indicated by # in Table 1.

**THE LENGTHS OF THE AERIAL PART AND THE PRIMARY ROOT OF NORMAL SEEDLINGS RESULTS**

<table>
<thead>
<tr>
<th>Dose of THX (mL)</th>
<th>Average</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerial part (cm)</td>
<td>Primary root (cm)</td>
<td>Aerial part (cm)</td>
</tr>
<tr>
<td>0</td>
<td>0.74 ± 0.02 a</td>
<td>1.80 ± 0.13 b</td>
<td>1.75 ± 0.02 c</td>
</tr>
<tr>
<td>75</td>
<td>0.71 ± 0.01 a</td>
<td>1.76 ± 0.16 b</td>
<td>1.64 ± 0.05 c</td>
</tr>
<tr>
<td>150</td>
<td>0.74 ± 0.01 a</td>
<td>1.79 ± 0.05 b</td>
<td>1.60 ± 0.04 d</td>
</tr>
<tr>
<td>225</td>
<td>0.76 ± 0.04 a</td>
<td>1.81 ± 0.17 b</td>
<td>1.52 ± 0.19 d *</td>
</tr>
<tr>
<td>300</td>
<td>0.78 ± 0.04 a</td>
<td>1.87 ± 0.14 b</td>
<td>1.47 ± 0.11 d *</td>
</tr>
<tr>
<td>Average</td>
<td>0.75</td>
<td>1.81</td>
<td>1.60</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>3.78</td>
<td>7.48</td>
<td>6.43</td>
</tr>
</tbody>
</table>

**Table 2. Length of the Aerial Part and the Primary Root Average**

<table>
<thead>
<tr>
<th>Dose of THX (mL)</th>
<th>Average</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerial part (cm)</td>
<td>Primary root (cm)</td>
</tr>
<tr>
<td>0</td>
<td>2.94 ± 0.09 f</td>
<td>5.85 ± 0.23 g</td>
</tr>
<tr>
<td>75</td>
<td>3.13 ± 0.05 f</td>
<td>5.71 ± 0.12 g</td>
</tr>
<tr>
<td>150</td>
<td>3.06 ± 0.15 f</td>
<td>5.69 ± 0.17 g</td>
</tr>
<tr>
<td>225</td>
<td>3.11 ± 0.30 f</td>
<td>5.64 ± 0.41 g</td>
</tr>
<tr>
<td>300</td>
<td>3.25 ± 0.15 f</td>
<td>5.94 ± 0.44 g</td>
</tr>
<tr>
<td>Average</td>
<td>3.10</td>
<td>5.77</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>5.55</td>
<td>5.28</td>
</tr>
</tbody>
</table>

Note: In the column, the average followed by the same lower-case letter does not differ statistically by the Scott-Knott test, 5% probability; the average followed by * differs statistically from the control group.

Source: Authors (2024)

On the third, fourth, and fifth days after the start of the test, the normal seedlings’ LA and LR were measured. Except for the average LA on the fourth day, where the doses of 150, 225, and 300 mL of THX significantly differed from the other groups according to the Scott-Knott test, these results did not show substantial differences. Furthermore, the averages of the treatment groups were not significantly different from the control group, except for the average of LA for the fourth day, which differs considerably from
the control group and is represented in Table 2 by the symbol *. Finally, Dunnett’s test revealed that the LA and LR group averages were not significantly different from the recommended dose on any of the days of analysis.

**SAND EMERGENCE RESULTS**

Table 3 shows the sand emergence average for the first count of emergence (FCE), the last count of emergence (LCE), and the speed of emergence index (SEI).

<table>
<thead>
<tr>
<th>Dose of THX (mL)</th>
<th>FCE average (%)</th>
<th>LCE average (%)</th>
<th>SEI average (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.75 ± 0.50 a</td>
<td>45.00 ± 0.00 a #</td>
<td>9.45 ± 0.05 a #</td>
</tr>
<tr>
<td>75</td>
<td>11.00 ± 1.41 a</td>
<td>44.00 ± 0.00 b *#</td>
<td>9.28 ± 0.07 b *#</td>
</tr>
<tr>
<td>150</td>
<td>11.75 ± 1.26 a</td>
<td>39.75 ± 0.50 c *</td>
<td>8.53 ± 0.12 d *</td>
</tr>
<tr>
<td>225</td>
<td>9.00 ± 0.82 b #</td>
<td>40.25 ± 0.50 d *</td>
<td>8.42 ± 0.05 d *</td>
</tr>
<tr>
<td>300</td>
<td>7.50 ± 1.00 b *#</td>
<td>43.00 ± 0.00 c *#</td>
<td>8.82 ± 0.06 c *#</td>
</tr>
<tr>
<td>Average</td>
<td>10.00</td>
<td>42.40</td>
<td>8.90</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>10.49</td>
<td>0.75</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Note: In the column, the average followed by the same lower-case letter does not differ statistically by Scott-Knott test, 5% probability; the average followed by * differs statistically from the control group, by Dunnett test, 5% probability; the average followed by # differ statistically from the group treated with 150 mL of THX, by Dunnett test, 5% probability.

Source: Authors (2024)

The FCE of the control group and groups treated with 75 and 150 mL doses showed higher average percentages of normal seedlings than the other groups. The Scott-Knott test revealed that these groups were significantly different from stressed groups. According to Nakagawa (1994), the groups with the highest vigor level presented higher percentages of normal seedlings in the FCE than the other groups. Besides, the most stressed group (treated with a 300 mL dose of THX) significantly differed from the control group by Dunnett's test, as indicated by the symbol * in Table 3. Moreover, the average percentages of stressed groups significantly differed from those treated with the recommended dose, as indicated by the symbol # in Table 3.

Scott-Knott test revealed that the group’s average percentages of LCE were significantly different. Furthermore, Dunnett’s test showed that the averages of the treatment groups differed significantly from the control group, as indicated by the symbol * in Table 3. In addition, Dunnett's test revealed that the average percentages of the control group and the groups treated with doses of 75 and 300 mL of THX were significantly different from those treated with the recommended dose, as indicated by the symbol # in Table 3.

Scott-Knott test showed that the control's average SEI significantly differed from the other groups. Furthermore, Dunnett’s test indicated that all treatment group averages
significantly differed from the control group, as indicated by the symbol * in Table 3. The Scott-Knott test revealed that the average SEI of the groups treated with 150 and 225 mL of THX was not significantly different. Once again, Scott-Knott’s test showed that these groups differed significantly from the control group and those treated with 75 and 300 mL of THX doses. Moreover, the average SEI of the group treated with 225 mL of THX was the only one that did not differ significantly from the recommended dose by Dunnett’s test, as indicated by the symbol # in Table 3.

The percentage FCE average strongly correlates with the total photon counts average, as shown in Table 4. The high rate of cell division and, thus, seed metabolism that occurs during initial seedling emergence could explain this strong correlation. Besides that, the average percentages of LCE and SEI correlate moderately and firmly with the photon counts average, respectively.

**Table 4 - Pearson’s correlation coefficient**

<table>
<thead>
<tr>
<th>Non-optical tests</th>
<th>FCE</th>
<th>LCE</th>
<th>SEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average of total photon count (x 10⁶) (arbitrary units)</td>
<td>0.90</td>
<td>0.41</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Source: Authors (2024)

**FTIR DATA RESULTS**

*Figures 3, 4, 5, 6, and 7 showed the PCA analyses of the absorption spectra for the five days to wheat seeds. Even though some samples formed clusters, PCA analyses of the absorption spectra did not allow us to identify variations as a function of treatment doses for the control group. One explanation is that the spectra differ little and cannot be*
The formation of clusters for specific doses on specific days was seen, but a pattern could not be determined. Here, it was noted that the results of the spectral analyses agreed with the averages of LA and LR of normal seedlings from the first to the fifth day after the test installation, where there were no observed significant differences between the groups.

CONCLUSIONS

Conventional methods can distinguish between seeds with varying physiological quality levels. However, these methods may need to provide more information about the physiological processes in wheat seeds. Because of this, the physiological quality of the seeds was examined in this study using optical techniques.

Despite numerous research studies, the physiological relevance of UWPEs has not yet been found; however, there is experimental evidence that this technique can be employed as a non-invasive diagnostic tool for monitoring processes in agriculture, such as abiotic and biotic stress effects on plants (POSPÍŠIL, 2014). Therefore, UWPE can be used to assess seed quality, a technique that uses non-complex equipment, can display results in real-time, and is non-invasive and nondestructive (NEMATOLLAHI et al., 2020). So, the UWPE technique can be used as an adjunct tool to study the physiological processes in wheat seeds. There is clear evidence that its results, particularly in the stressed groups of seeds, had a link with the FCE and SEI in non-optical testing. This feature may be related to probable reductions in the seeds' early metabolisms. Nonetheless, the findings of normal seedlings at the LCE average indicated that these potential metabolic deficits were somewhat restored. Furthermore, a rise over time was detected in the study of the temporal profiles of the UWPEs; however, the rates were lower in the most stressed groups than in the other groups.

The PCA analysis performed on the daily absorption spectra did not allow the different seed groups to be categorized into distinct clusters. These findings are based on readings of the averages of LA and LR of normal seedlings performed from the third to the fifth post-installation of the tests, which were not sensitive enough to detect possible differences in the vigor level of wheat seeds submitted to different THX doses compared to the control group.

Therefore, the results of the optical techniques are anticipated to be used to analyze the effects of TXH on the physiological quality of wheat seeds, assist in decision-making, and supplement the data provided by standard testing.
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