

**SUPPORTING
DATA AND EVIDENCE
FOR THE PROPOSED RULES
CHANGES 2005 –
ITEM 4B**

**FOR INFORMATION AND CONSIDERATION AT THE
ORDINARY MEETING 2005**





**SUPPORTING DATA AND EVIDENCE FOR PROPOSED RULES CHANGES - ITEM 4B:
TO INCLUDE COMPOST AS A PRIMARY SUBSTRATE FOR SUNFLOWER GERMINATION**

COMPARATIVE TEST IN SUBSTRATES FOR SUNFLOWER GERMINATION

**Report of the Results and Statistical Analysis by
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Use of compost as a primary substrate for the germination of Sunflower seeds

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In order to decide if compost can be used as primary substrate for sunflower germination, a comparative test between 15 seed laboratories applying ISTA or AOSA methods was organised.

The ISTA Method Validation Program was used at all stages of the comparative test from the planning of the test to the elaboration of the protocol and the analysis of the results. The comparative test involved germination tests being conducted on 5 samples of Sunflower, at the temperature of 20°C, with the 3 substrates: paper, sand and compost.

Statistical analysis of the results shows that the number of normal seedlings is highest with the use of compost compared to the 2 other substrates. This is principally due to a decrease of abnormal seedlings (root defects and defects of the whole seedling) and to a less extent to a decrease of non germinated seeds. Germination in sand results in more ungerminated seeds compared to germination in paper and compost. On the other hand, germination using paper substrate results in more abnormal seedlings compared to germination in compost and sand.

Repeatability and reproducibility of the results are also improved with compost than with paper or sand. Within participating laboratories the use of compost results in higher or equal germination results compared to the method used as a routine in each laboratory, even if the laboratory is not experienced with compost. On the contrary, germination using sand or paper can give lower results if the laboratory is inexperienced in the use of either media.

As a result of this comparative test it is recommended that compost can be used as a primary substrate for Sunflower germination.



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Introduction

In 2001, at the ISTA Congress in Angers, the Germination Committee of ISTA decided to set up a Working Group dealing with “Substrates and temperatures for the germination of Sunflower” in order to harmonise the rules between AOSA and ISTA. At the Congress, FIS and GEVES (France) reported data from different laboratories comparing germination with different substrates including compost. The data demonstrated that germination results, in general, were higher when compost was used as a germination medium compared to sand or paper (unpublished).

The prime objective of the Sunflower Working Group was to establish whether compost is a suitable germination medium for Sunflower germination and whether it could be proposed to be included in the ISTA Rules as a primary substrate for Sunflower germination. A search of the literature on Sunflower germination revealed no references regarding the use of compost substrate or the comparison of compost to other substrates. One study by Cseresnyes (1979)¹ reported the results of the effect of two different substrates, rolled towel and sand, on the germination of Sunflower seeds. It demonstrated that there was no significant difference between these 2 substrates on the results of germination, at 25°C, of 64 samples of Sunflower.

The anecdotal evidence suggesting that compost media resulted in higher germinations of Sunflower seedlots and the lack of supporting evidence in the literature resulted in the organisation of this comparative test. It involved ISTA and AOSA laboratories and compared three types of substrates for Sunflower germination : paper; sand; and compost.

The ISTA Method Validation Program was followed throughout this comparative test.

Protocol of the comparative test

The protocol of the comparative test was defined after analysis of answers of participants to a questionnaire regarding: their experience of Sunflower germination; the use of ISTA/AOSA Rules; and their experience in using paper, sand and compost germination media. The protocol was has been established by S. Grégoire, chairman of ISTA Statistical Committee, and validated by two technical experts from the Germination and Tetrazolium Committees, G. Tarp and A. Martinelli.

¹ Cseresnyes Z. (1979). The germination of *Helianthus annuus* under optimum laboratory conditions. Seed Science and Technology, 7, 319-328.



The protocol involved :

16 participating **laboratories**

- 10 using ISTA methods
- 6 using AOSA methods

All the laboratories involved in the comparative test to have experience of sunflower germination testing. They also had to have experience of germination testing using at least one of the substrates being investigated (paper, sand or compost).

5 **samples** of Sunflower seeds to be used, 2 of which were to be (intentionally) the same (prepared from the same seed lot)

Germination methods : 3 germination methods to be used for each sample

- The usual ISTA germination method of the laboratory (sand or paper)
- Germination method with another substrate (sand or paper)
- Germination method with compost

- One common temperature to be used for all the tests : 20°C

- Germination tests to be carried out on 4 replicates of 100 seeds.

- Each germination method to be repeated 2 times for each sample in order to differentiate variability inside laboratory and variability between laboratories

Germination results to be reported by two different ways:

- On sheets giving numbers of normal seedlings, abnormal seedlings and ungerminated seeds (fresh and dead seeds). See data in Annexe E.
- On sheets giving details of the types of abnormal seedlings found abnormal seedlings were separated by organ affected. For root systems, defects were separated in 2 classes : A- Primary root defective with sufficient secondary roots; B- Primary root defective without sufficient secondary roots. See data in Annexe F.

Analysis of the comparative test to include:

1. Results of repeatability, intra laboratory variability (within)
2. Results of reproducibility, inter laboratory variability (within + between)
3. Results of a comparison germination in compost media to existing approved ISTA and AOSA methods, i.e. a comparison of results with compost substrate to results with paper and sand substrates.



Rational and justification for the statistical design and analysis of the Comparative test investigating the effect of substrate on the germination of Sunflower in ISTA and AOSA laboratories

Experimental Design

At time of planning the experiment the key issues of the study were to:

- Compare three substrates (sand, paper and compost);
- Check and quantify repeatability and reproducibility of tests; and
- Check if ISTA and AOSA rules give similar test results.

In order to be able to check the global validity of the results before considering the key issues, it was decided:

- To include a duplicate sample within the trial. In other words 2 samples from one seedlot were included in the samples sent to participants in order to check if results from these two samples were similar as expected; and
- To ask participants to carry out 2 separate germination test on each sample, in order to check if results from these two assays were similar as expected. By repeating each modality in this way information is also available on intra-laboratory variability.

The Data and the Analysis

Data was thoroughly checked prior to any analysis by sending it back to participants for formal validation.

The data expected from individual tests have values from 0 to 100 for different variables (normal seeds, fresh seeds, ...) and it is generally assumed that the results from germination tests follow a binomial distribution. However, when the number of seeds is large enough (as in this test 100 for each replicate) and the p parameter is not too close to 1 or 0, the Normal approximation of the Binomial can be used. The distribution of data and its variability has also been examined in order to check validity of normal approximation. From this check it was recommended that fresh seed should not be analysed in isolation because of their negligible numbers and it was decided to sum fresh and dead seeds.

When considering the validity of the data and the analysis it was decided that:

- In case of unexpected differences in general, the data would not be computed to check the key issues.
- In case of unexpected differences for a given laboratory, the data from this laboratory would not be included in the analysis.
- In case of unexpected differences for a given modality (for instance in a laboratory for a given substrate), the corresponding data would be removed from the analysis.



These checks were performed prior to the analysis of the key issues, and after a thorough check of the raw data (formal validation by participants). Assuming the Normal approximation of the Binomial distribution for the results allows the use of ISO 5725-2, as well as analysis of variance in order to study the effects of the experiment (laboratory, substrate, sample, ISTA/AOSA) and to compare means.

ISO 5725-2 was used for the analysis since it is now considered the reference method for quantifying and checking repeatability and reproducibility when results are quantitative. Analysis of variance is a well known technique, which is robust and easy to interpret. Both Analysis of Variance and ISO 5725-2 can be performed in any laboratory having computer facilities, which is not the case for some other techniques where expensive or specific software is required.

For all aspects (factor effects, repeatability, reproducibility) it was decided to use graphs in the report rather than tables, in order to ease understanding and interpretation of results. The corresponding tables of data (Analysis of variance, etc...) were produced and used to draw the conclusions made in the report.

Comments on the results and the methods

Results

We received 15 sets of results from the 16 laboratories that received samples for the comparative test. We did not receive the results from lab 13.

The methods that were used by the 15 laboratories were listed in tables 1, 2 and 3 in [Annexe A](#). The report of the methods used from all the participating laboratories allowed checks to be made regarding their compliance with the protocol. These checks revealed that:

- Laboratory n°15 had not performed germination tests in sand and compost (it only reported results using paper substrate).
- Laboratory n° 4 did not performed germination tests in paper substrate (it only reported results using sand and compost substrates).
- 3 laboratories had not conducted the tests at 20°C as required:
 - Laboratories n°s 2 and 12 used a temperature of 25°C
 - Lab n° 4 used temperature of 20-30°C

In conclusion 11 laboratories applied the correct protocol (they conducted germination tests using the 3 substrates (sand, paper and compost) at a temperature of 20°C).



Comparison of substrates used by participating Laboratories

➤ Germination methods with **SAND**

- **Size composition** is quite similar between laboratories (maximum size varies between 0,4 to 1,0 mm)
- **Type of sand** is silver sand or river sand
- **Water content** differs between laboratories. Percentage of water on a dry weight basis varies between 5 to 35%.

➤ Germination methods with **PAPER**

- **Type of paper** : pleated paper or rolled towel
- **Water content** varies greatly between laboratories from 70 to 352% of the dry weight of the paper

➤ Germination methods with **COMPOST**

- **Composition** differs between laboratories (see Annexe A)
 - . peat (2 laboratories used peat : one lab used 100% Baltic up land peat; the other used peat and nutrients)
 - . peat + soil (1 lab used a mixture of 33% peat – 67% artificial soil)
 - . peat + sand (8 laboratories used a mixture peat and sand : 5 with 80% peat – 20% sand; 1 with 82% peat – 18% sand; 1 with 95% peat – 5% sand; 1 with no precise proportions)
 - . peat + Perlite (3 laboratories used a mixture of peat and Perlite : 1 with 80% - 20% proportion, 1 with no precise proportions, 1 with 75% peat – 25 % Perlite, Dolomitic limestone, gypsum)Nutrients have been added to some composts.
- **Water content** varies greatly between laboratories from 3 to 300% of the compost dry weight

In summary, the composition and water content of the substrates used by participating laboratories varies greatly between the laboratories. These differences appear to be smaller with sand and greater with paper and compost.

Repeatability intra laboratory (Within laboratory)

The analysis of repeatability was carried out on the results from the 11 laboratories that followed the protocol and the results of the analysis of normal seedlings only are presented.

1. Results by substrate

Repeatability was analysed using the computer program developed by S. GREGOIRE (2002) based on the ISO 5725-2 definitions.

k values are indicator on how repeatable repeat tests were. Each bar of the histograms below is the k value for a sample in a lab for the given sample. The 5 sample values are grouped by laboratory (laboratory code on the Y-axis). A k value of zero would indicate that repeat tests gave exactly the same result as the original tests. The greater the k value is, the less repeatable the results are.

ISO 5725-2 gives a table with critical values. The critical value depends only on the number of laboratories, and the number of repeats per sample tested.

When a k value exceeds this critical value, it is an indication that for this sample in this lab, the variability is significantly different of the average variability.

This helps to check consistency and identify outliers.

When a k value exceeds the critical limit a check is made to confirm that the results reported by the laboratory are free of mistakes (i.e. a typing/writing error). If the results are confirmed the results are included in the analysis and the values reported.

▪ SAND Substrate

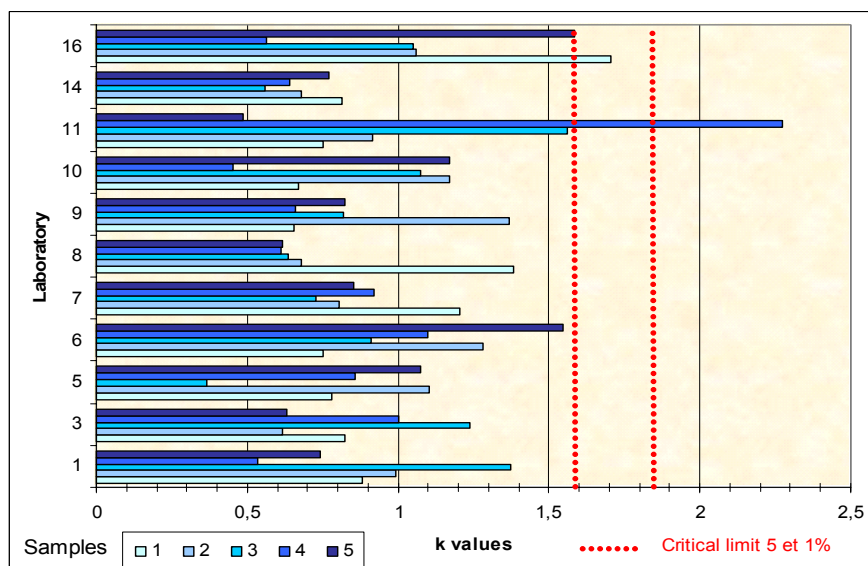


Figure 1 : repeatability (k values) obtained by the 11 laboratories that followed the correct protocol. Results are presented by laboratory for the 5 sunflower samples tested, for sand substrate.

Two laboratories have k values exceeding the critical level : laboratory 11 for sample 4 (Probability < 1% level) and laboratory 6 for sample 1 (Probability > 1% but < 5% level).

▪ **PAPER substrate**

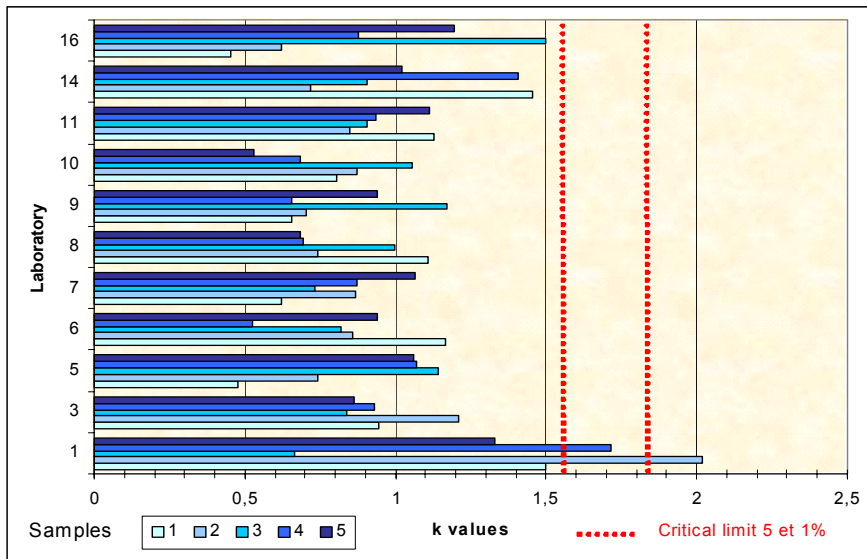


Figure 2 : repeatability (k values) obtained by the 11 laboratories that followed the correct protocol. Results are presented by laboratory for the 5 sunflower samples tested, for paper substrate.

One laboratory has k values exceeding the critical level for two samples: Laboratory 1 for sample 2 (Probability < 1% level) and sample 4 (Probability > 1% but < 5% level).

▪ **COMPOST substrate**

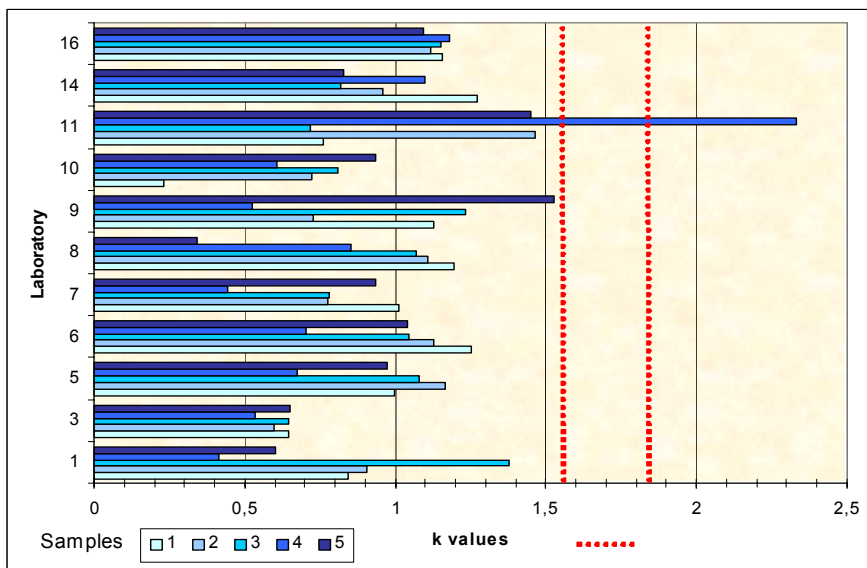


Figure 3 : repeatability (k values) obtained by the 11 laboratories that followed the correct protocol. Results are presented by laboratory for the 5 sunflower samples tested, for compost substrate.

Two laboratories have k values exceeding the critical level: laboratory 11 for sample 4 (Probability < 1% level) and laboratory 9 for sample 5 (Probability > 1% but < 5% level).

2. Results by germination quality

Results of the standard deviation for repeatability (sr2) obtained by the computer program (GREGOIRE S., 2002) have been plotted versus the germination quality (normal seedlings) of the samples. Results for abnormal seedlings and ungerminated seeds are in **Annexe B**.

Repeatability (sr2 in ISO 5725-2) is an indicator of within laboratory consistency of results. On the following graphs the mean germination value of all laboratories for a given sample is plotted on the X-axis, and the sr2 value on the Y-axis. The lower the sr2 value is, the more repeatable the results are. It should be noted that in calculation sr2 values all results were computed including those where repeatability problems were identified though the calculation of k values

The 3 substrates are compared in Figure 4.

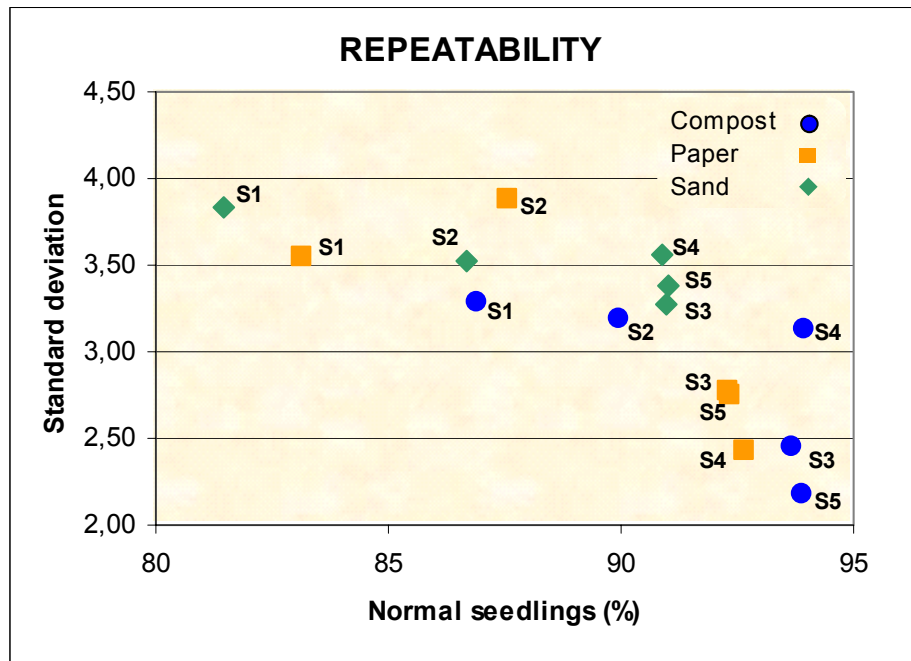


Figure 4 : Variation of repeatability towards germination quality of the samples for all the substrates tested.

From Figure 4 it is clear that repeatability improves with increases in germination and that the maximum repeatability is obtained when compost media is used.

3. Results by Sample tested

Repeatability has also been computed for the 5 samples tested on the 3 different substrates. These are presented for the normal seedlings (abnormal seedlings and ungerminated seeds are in **Annexe B**). For this computation the results with significant k values were excluded from the calculation of sr2.

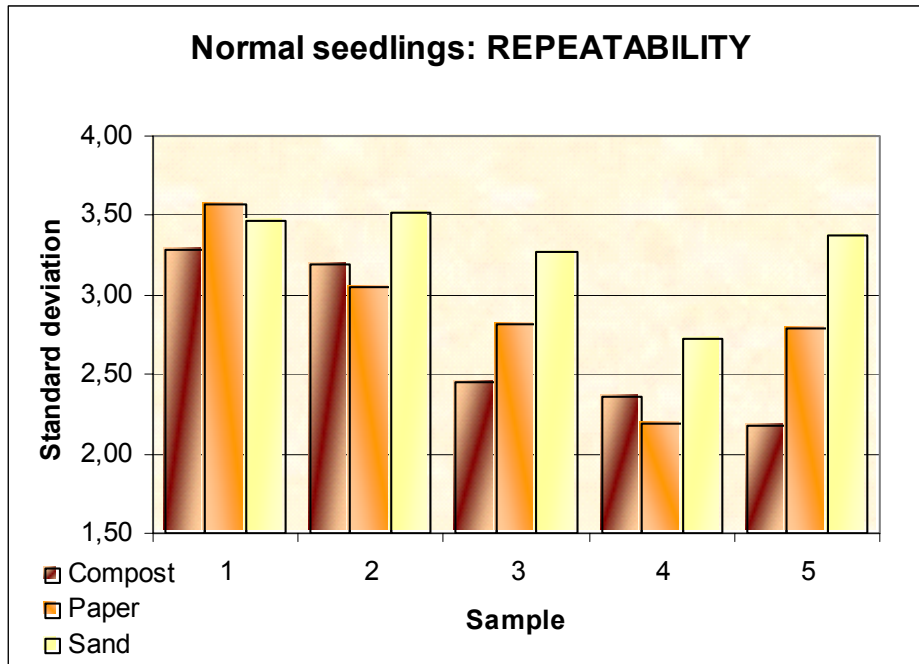


Figure 5: results of repeatability by sample and by substrate for the percentage of normal seedlings.

From Figure 5 it is clear that compost gives the best repeatability for Samples 1, 3 and 5. Sand gives the lowest level of repeatability except for sample 1. The repeatability obtained when using paper is intermediate: it is better than that obtained in sand tests except for sample 1 and it is worse than that obtained in compost tests except for samples 2 and 4.

The mean sr2 values over all samples are presented in Figure 6.

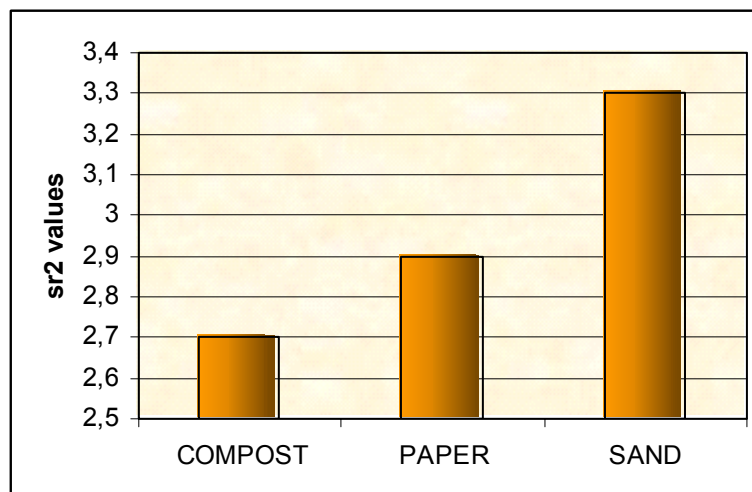


Figure 6 : Mean values of sr2 on all the samples

This Figure confirms that when comparing the 3 substrates (paper, sand and compost) repeatability is best in the compost tests. It is also clear of the other substrates repeatability is better in paper tests than in sand tests.

Reproducibility inter laboratory (between laboratories)

Results of reproducibility are presented for normal seedlings only. The analysis was performed on the results from the 11 laboratories that followed the protocol.

1. Results by substrate

Reproducibility was analysed with the computer program developed by S. GREGOIRE (2002) based on the ISO 5725-2 definitions.

h values are indicator whether over-estimation or under-estimation occurs and each bar of the histograms below is the h value for a given sample tested in a particular laboratory. The reference used to calculate the individual h values is the average germination from all laboratories for a particular substrate. A negative h value indicates that the value obtained is below the average, a positive h value indicates that the value obtained is over the average.

ISO 5725-2 gives a table with critical values. The critical value depends only on the number of laboratories, and the number of repeats per sample tested. When a h value exceeds this critical value, it is an indication that for this sample in this particular laboratory, the over ($h > 0$) or under ($h < 0$) estimation is significantly different from the average.

In this comparative test the true value of the samples is not known and it is not possible to ascertain whether the average of all results is a good reference. Consequently in this comparative test, h values are a good tool to scrutinise for consistency, and to visualise laboratories that over or under estimate in comparison to the mean results of all laboratories, but there is no reason to discard values that have a significant h value.

In the following graph looking at the influence of germination substrate the 5 samples values are grouped by laboratory (laboratory code on the Y-axis).

▪ SAND Substrate

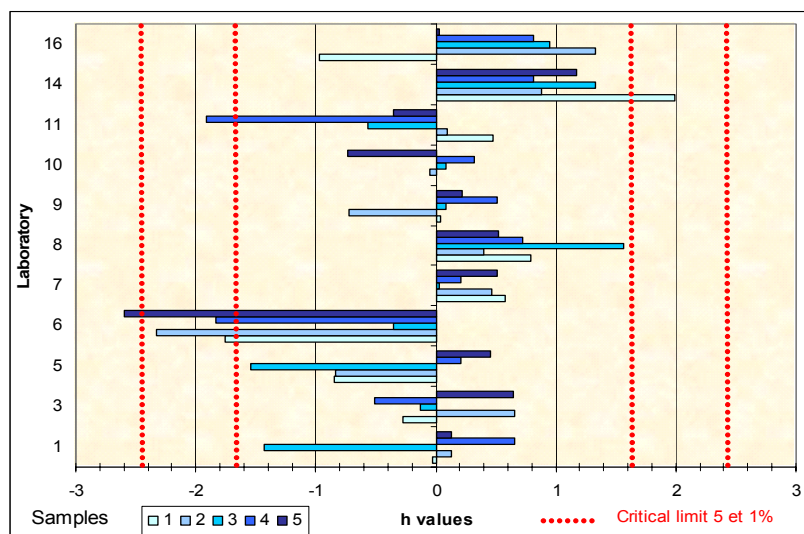


Figure 7 : reproducibility (h values) obtained by the 11 laboratories that followed the correct protocol. Results are presented by laboratory for the 5 sunflower samples tested, for sand substrate.

Results of laboratory 6 differ from the results of the general (0-value) mean with sand substrate. This laboratory shows a tendency to find less normal seedlings than the other laboratories, especially for sample 5 (1% level) and samples 1, 2, 4 (5% level). Laboratory 11 finds less normal seedlings than the general mean for sample 4 (5% level). On the other hand, laboratory 14 finds more normal seedlings than the general mean for sample 1.

▪ **PAPER substrate**

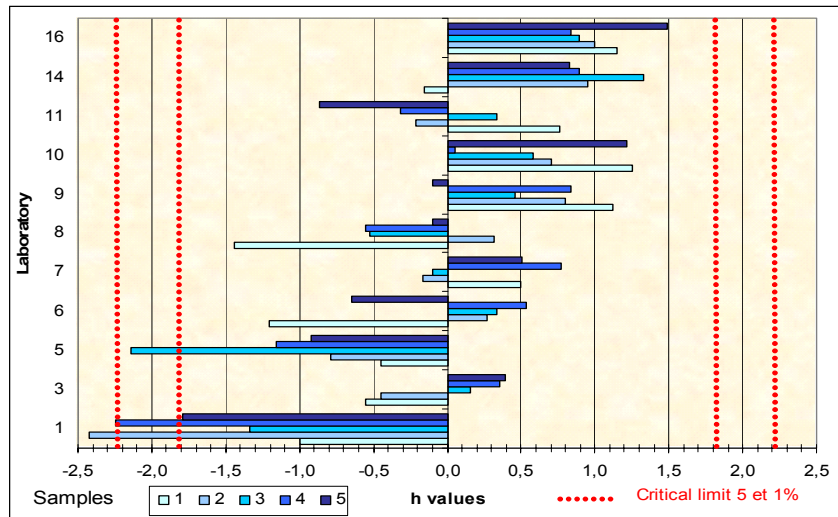


Figure 8 : reproducibility (h values) obtained by the 11 laboratories that followed the correct protocol. Results are presented by laboratory for the 5 sunflower samples tested, for paper substrate.

Results of laboratory 1 differ from the results of the general (0-value) mean with paper substrate. This laboratory shows a tendency to find less normal seedlings than the other laboratories, especially for sample 2 and 4. Laboratory 5 has the same tendency especially for sample 3 (5% level).

▪ **COMPOST substrate**

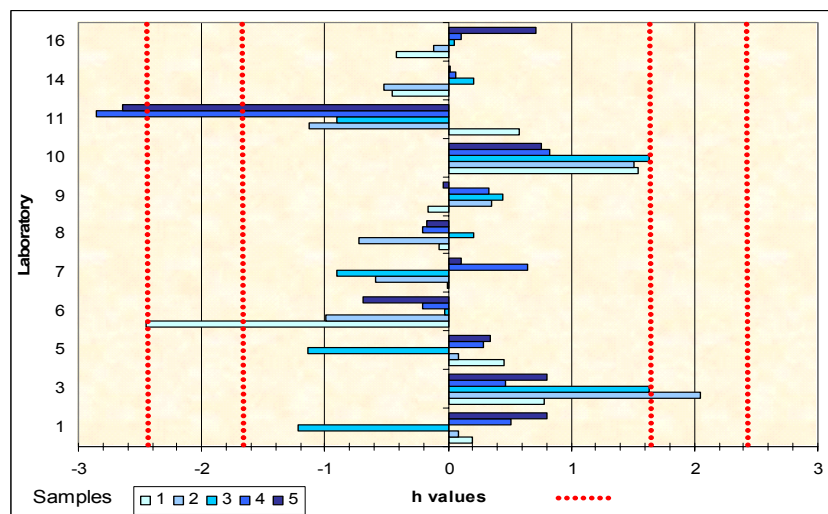


Figure 9 : reproducibility (h values) obtained by the 11 laboratories that followed the correct protocol. Results are presented by laboratory for the 5 sunflower samples tested, for compost substrate.

Results of laboratory 11 differ from the results of the general (0-value) mean with compost substrate. This laboratory shows a tendency to find less normal seedlings than the other laboratories, especially for sample 4 and 5 (1% level). On the other hand, laboratory 3 finds more normal seedlings than the general mean for sample 2 (5% level).

2. Results by germination quality

Results of the standard deviation for reproducibility (sR2) obtained by the computer program (GREGOIRE S., 2002) have been plotted versus germination quality (normal seedlings) of the samples. Results for abnormal seedlings and ungerminated seeds are in [Annexe C](#).

The 3 substrates can be compared on the following Figure.

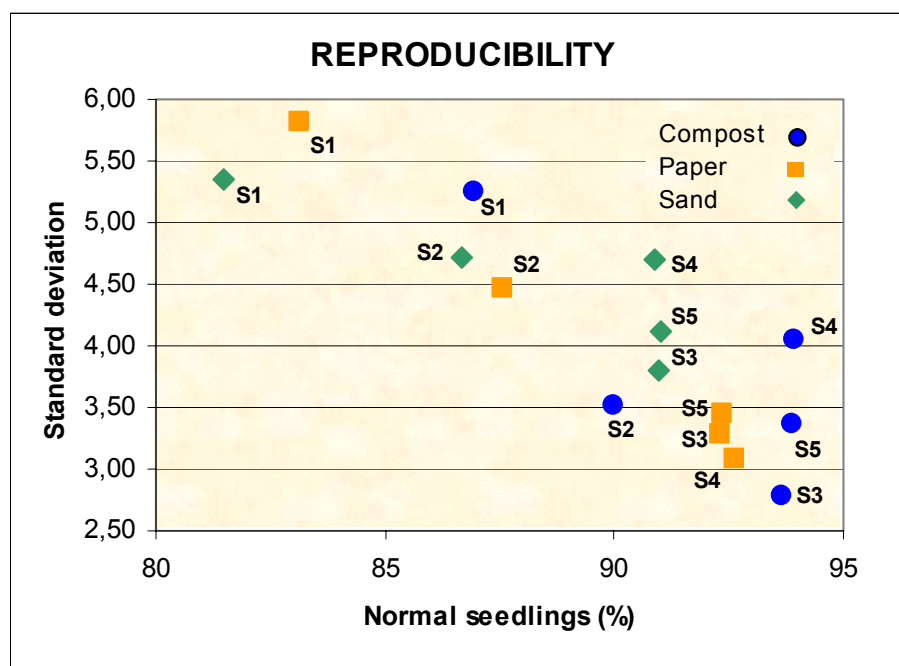


Figure 10 : Variation of reproducibility versus germination quality of the samples for all the substrates tested².

Figure 10 shows that reproducibility is best when the germination level is highest. In terms of substrates reproducibility of the results is similar for compost and paper which are better, in terms of reproducibility, than sand.

² All data were analysed, even if significant k values were obtained.

3. Results by Sample tested

Reproducibility has been compared for the 5 samples tested on the 3 different substrates and the results for the normal seedlings are presented in Figure 11 (abnormal seedlings and ungerminated seeds are in [Annexe C](#)).

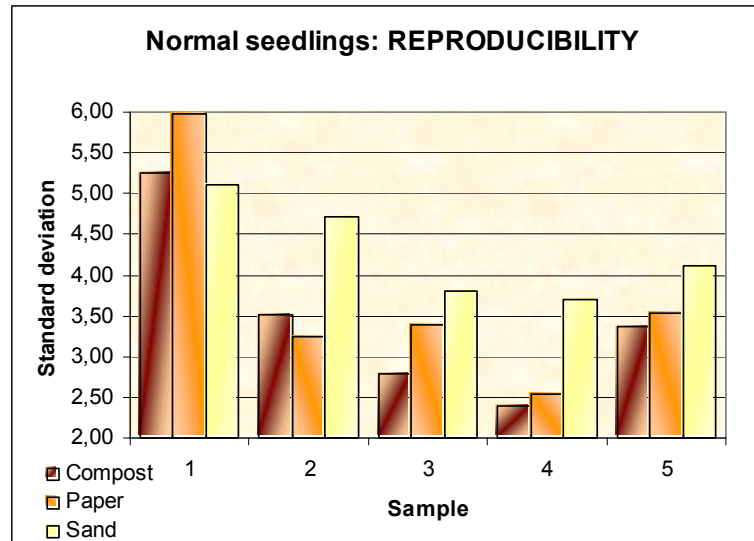


Figure 11 : results of reproducibility by sample and by substrate for the percentage of normal seedlings³.

From Figure 11 it is clear that compost gives the best reproducibility for Samples 3, 4 and 5. Sand gives the lowest level of reproducibility except for Sample 1. The reproducibility obtained when using paper is intermediate: it is better than that obtained in sand tests except for Sample 1 and it is worse than that obtained in compost tests except for Samples 2.

The Mean values of sr2 over all the samples are presented in Figure 12.

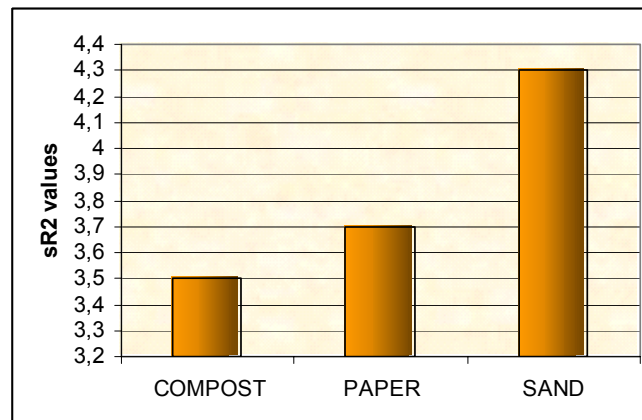


Figure 12 : Mean values of sr2 on all the samples

This Figure confirms that when comparing the 3 substrates (paper, sand and compost) reproducibility is best in the compost tests. It is also clear that of the other substrates reproducibility is better in paper tests than in sand tests.

³ Where significant k values were obtained for individual samples in individual laboratories the results were not .

Comparison of the compost to the paper and sand substrate

The results obtained from all the laboratories which performed germination tests using the 3 substrates including the results of laboratories who used a temperature regime differing from that dictated by the protocol (i.e. constant 20°C) are given in **Annexe D**. In this report however, the comparison of substrates is based on the results obtained by the eleven laboratories that followed exactly the protocol prescribed. Using the STATGRAPHICS Software the results from these 11 laboratories were subject to Variance Analysis..

Analysis of variance was carried out on each category of seedlings and seeds (normal seedlings, abnormal seedlings and ungerminated seeds (ie fresh plus dead seeds)). Since there were large differences between laboratories regarding the level of abnormal seedlings, analysis of variance has also been performed for some categories of abnormal seedlings: root defects and seedling as a whole.

Very low numbers of fresh seeds were reported by 7 of the laboratories. The others have found no fresh seeds regardless of the substrate used (see results in Annexe E). The level of fresh seeds was completely dependent of the laboratory testing the seed and decreasing numbers of fresh seeds was usually compensated for by increasing numbers of dead seeds. For this reason it was decided that fresh seed should not be treated as a separate category and to include them with the dead seed in a “fresh + dead seeds” category.

Results of the analysis are presented by category of seedling and seed and the following are presented for each category:

- The comparison of substrates, all samples together
- The comparison of substrates, for ISTA and AOSA laboratories
- The comparison of substrates, for each sample (substrate * sample interaction)
- The comparison of substrates, for each laboratory (substrate*lab interaction)

1. Normal seedlings

1.1. Comparison of substrates, all samples together

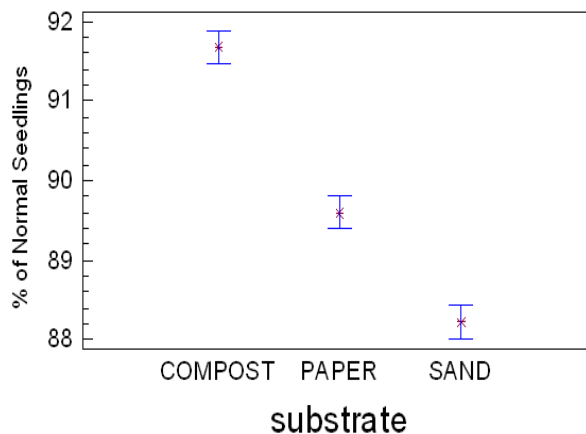


Figure 13 : Mean and 95% intervals for % of normal seedlings, for all samples, for each substrate

Figure 13 clearly demonstrates that normal seedlings are significantly higher in compost tests than in sand or paper tests. Furthermore, it is clear that sand tests give significantly lower numbers of normal seedlings than tests carried out using compost or paper media.

1.2. Comparison of substrates, for ISTA and AOSA laboratories

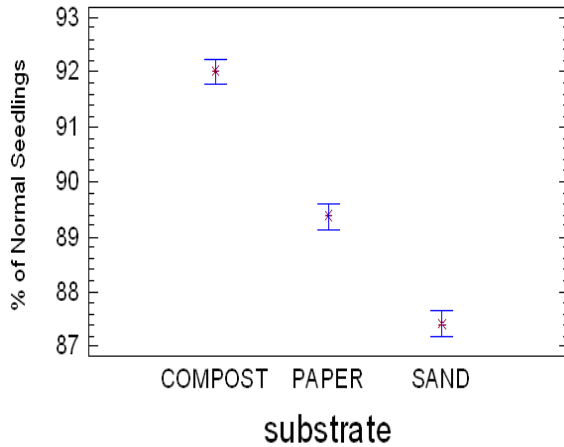


Figure 14 : Mean and 95% intervals for % of normal seedlings, for all samples, for each substrate, for ISTA laboratories only.

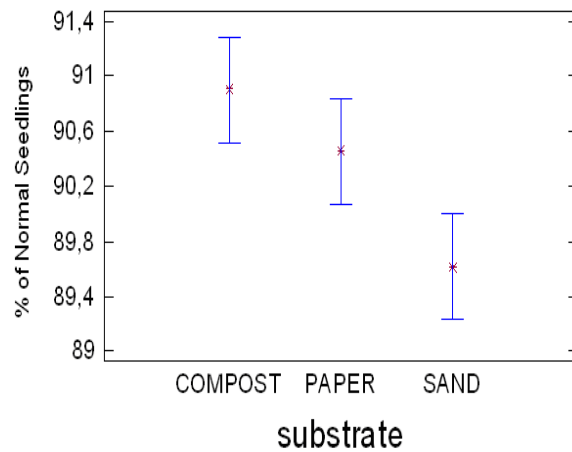


Figure 15 : Mean and 95% intervals for % of normal seedlings, for all samples, for each substrate, for AOSA laboratories only.

As with the overall findings, when laboratories were considered as a whole, there are significant differences between the 3 substrates when ISTA laboratories are considered separately (Figure 14) with highest germinations being obtained with compost media and lowest germinations being obtained in sand. Paper media is again intermediate giving significantly higher germinations than sand but significantly lower germinations than compost. For AOSA laboratories (Figure 15) there is no significant difference between the number of normal seedlings obtained in compost and paper germination tests but sand tests produce significantly lower numbers of normal seedlings than paper or compost tests.

1.3. Comparison of substrates, for each sample (interaction substrate * sample)

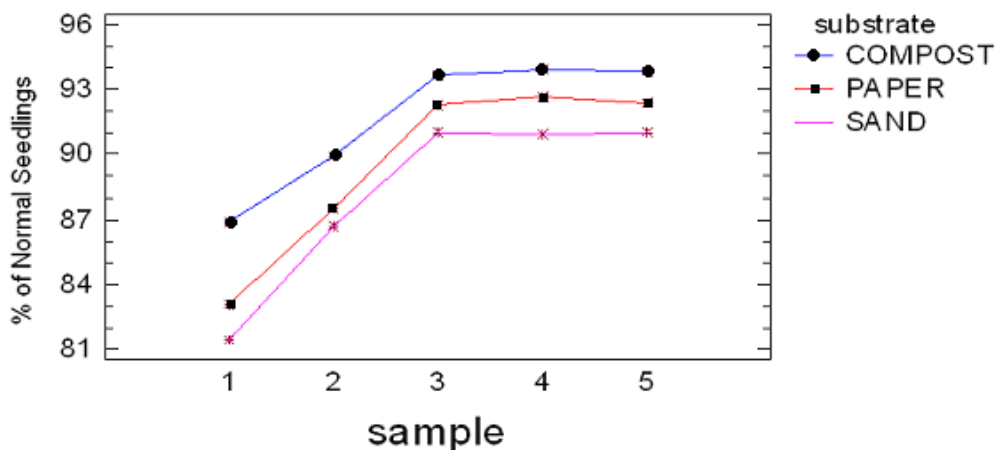


Figure 16: % of normal seedlings for each sample and for each substrate.

Irrespective of the sample the percentage of normal seedlings is always higher in compost tests, compared to paper and sand tests, and the level of normal seedlings is higher in paper tests than in sand tests.

1.4. Comparison of substrates, for each laboratory (interaction substrate * lab)

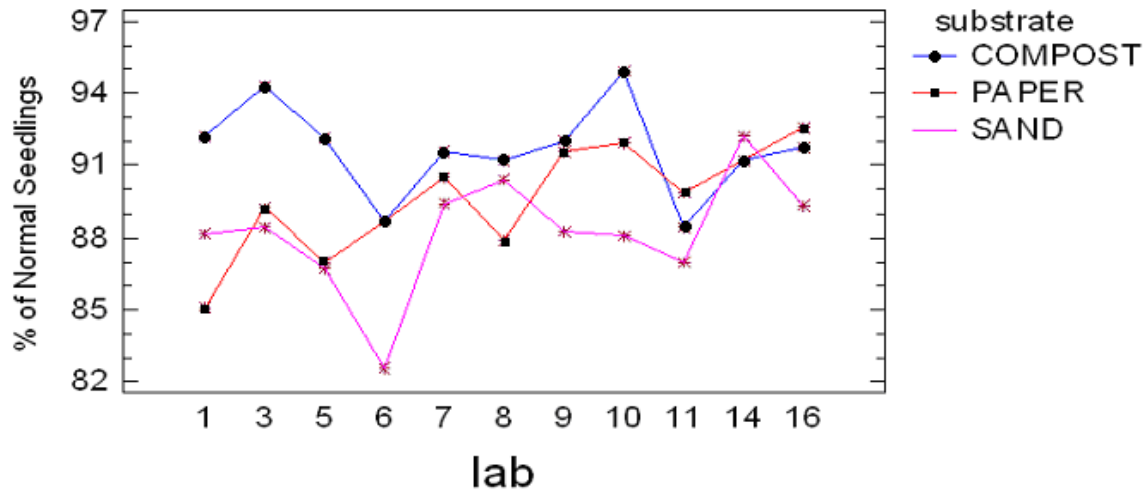


Figure 17: % of normal seedlings for each laboratory and for each substrate.

Looking at the results produced by the individual laboratories the percentage of normal seedlings is always higher with compost for laboratories 1, 3, 5, 6, 7, 8, 9 and 10. For laboratories 11 to 16, compost gives lower or equal results as paper.

2. Ungerminated seeds (fresh + dead seeds)

2.1. Comparison of substrates, all samples together

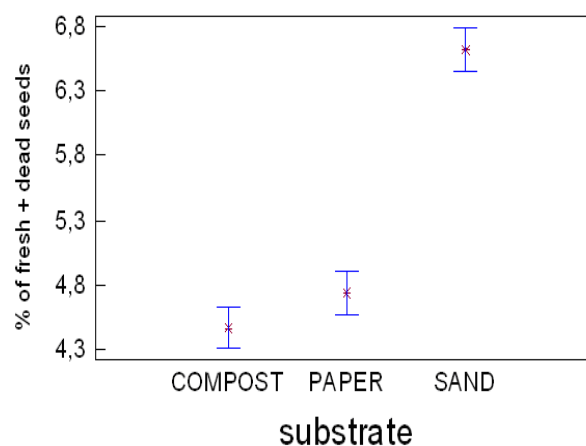


Figure 18 : Mean and 95% intervals for % of ungerminated seeds, for all samples, for each substrate

Over all laboratories there is no significant difference in the levels of ungerminated seeds found in compost and paper tests (Figure 18). On the other hand, sand tests result in a significantly higher level of non germinated seeds than paper or compost tests.



2.2. Comparison of substrates, for ISTA and AOSA laboratories

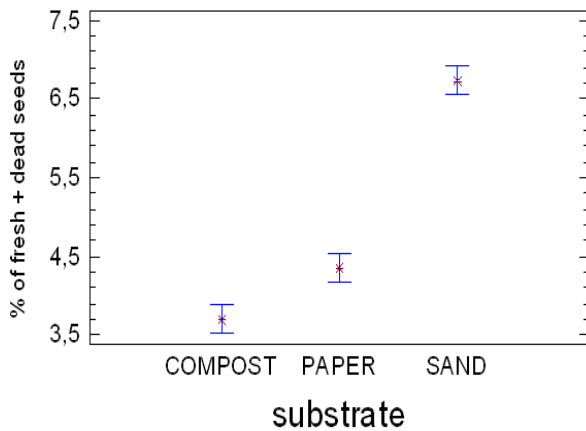


Figure 19 : Mean and 95% intervals for % of ungerminated seeds, for all samples, for each substrate, for ISTA laboratories only.

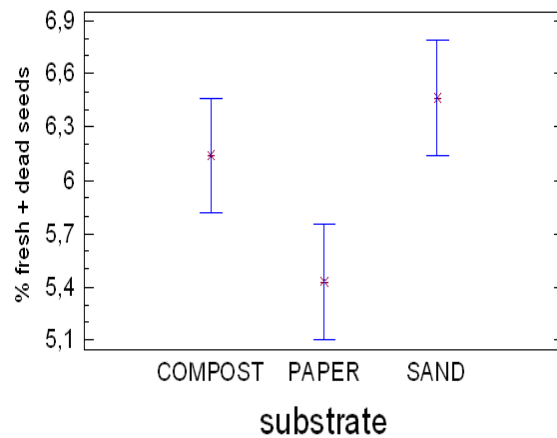


Figure 20 : Mean and 95% intervals for % of ungerminated seeds, for all samples, for each substrate, for AOSA laboratories only.

Unlike the findings, when laboratories were considered as a whole, there are significant differences between all the 3 substrates when ISTA laboratories are considered separately (Figure 19) with highest numbers of ungerminated seeds being obtained with sand media and lowest being obtained in compost. Paper media is intermediate giving significantly higher numbers of ungerminated seeds than compost but significantly lower numbers than sand. For AOSA laboratories (Figure 20) there is no significant difference between the number of ungerminated seeds obtained in compost and sand germination tests but paper tests produce significantly lower numbers of ungerminated seeds than sand or compost tests.

2.3. Comparison of substrates, for each sample (interaction substrate * sample)

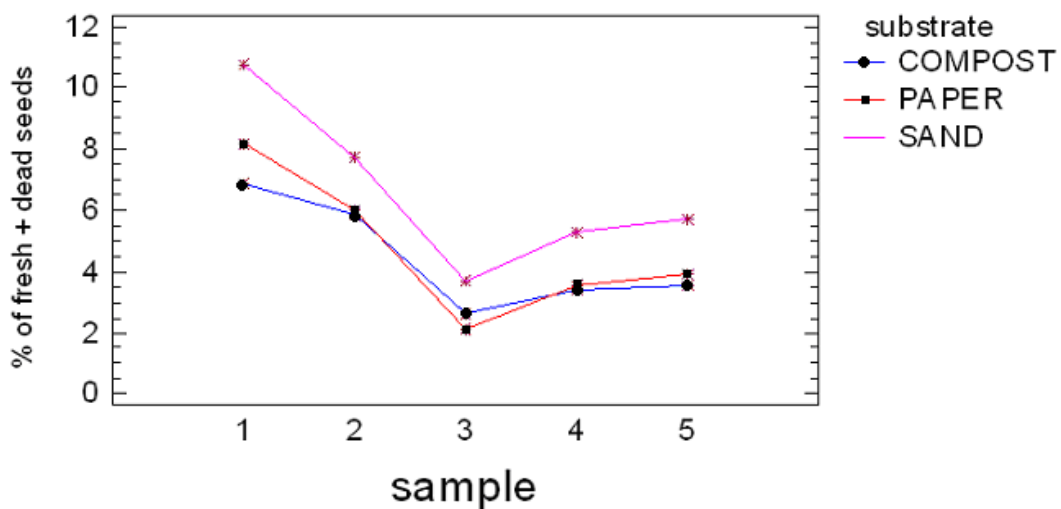


Figure 21: % of ungerminated seeds for each sample and for each substrate.

For all samples the highest numbers of ungerminated seed are found in sand tests (Figure 21) with paper and compost tests producing similar levels of ungerminated seeds.

Comparison of substrates, for each laboratory (interaction substrate * lab)

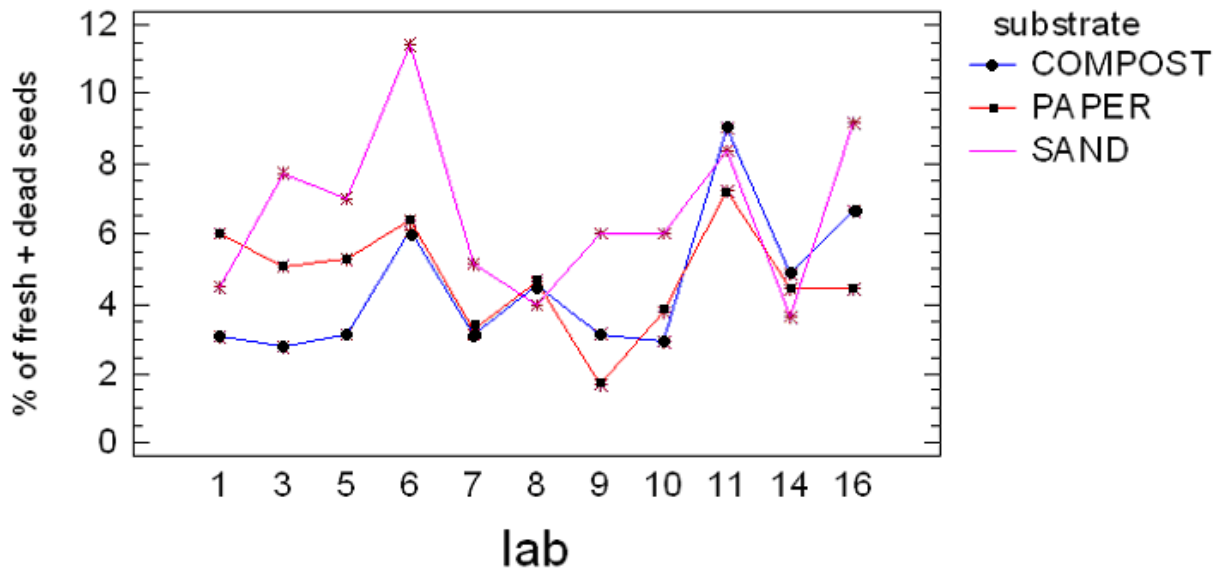


Figure 22 : % of normal seedlings for each laboratory and for each substrate.

When considering the results from individual laboratories, (Figure 22) it was found that the percentage of ungerminated seeds is generally higher with sand substrate except for laboratories 1, 8, 11 and 14. For laboratory 1 the percentage of ungerminated seeds was higher with paper substrate and for laboratories 8, 11 and 14 the percentage was higher with compost.

3. Abnormal seedlings

3.1. Comparison of substrates, all samples together

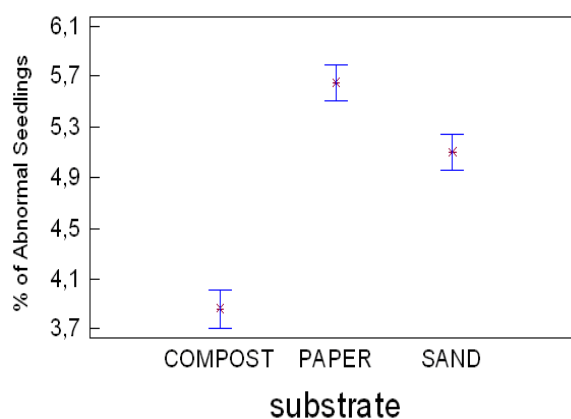


Figure 23 : Mean and 95% intervals for % of abnormal seedlings, for all samples, for each substrate

There are significant differences between the percentage of abnormal seedlings obtained with the 3 substrates (Figure 23). Over all laboratories paper substrates generates significantly higher numbers of abnormal seedling than either sand or compost. Compost media gives the lowest level of a abnormal with sand giving intermediate numbers.

3.2. Comparison of substrates, for ISTA and AOSA laboratories

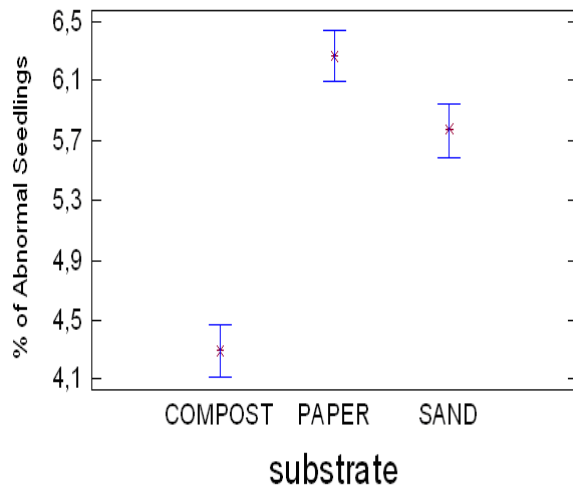


Figure 24 : Mean and 95% intervals for % of abnormal seedlings, for all samples, for each substrate, for ISTA laboratories only.

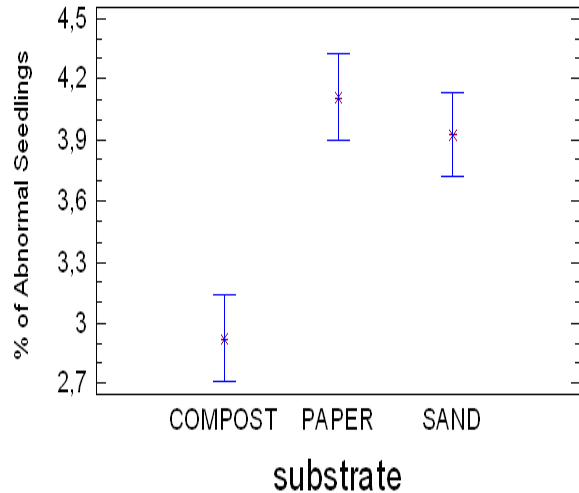


Figure 25 : Mean and 95% intervals for % of abnormal seedlings, for all samples, for each substrate, for AOSA laboratories only.

When the results of ISTA laboratories are examined separately (Figure 24), the 3 substrates give significant different levels of abnormal seedlings. Compost tests give the lowest percentage of abnormal seedlings and paper media generates highest percentage of abnormal. Results from AOSA laboratories are similar (Figure 25) but for AOSA laboratories difference between the levels of abnormal seedlings in paper and sand is not significant.

3.3. Comparison of substrates, for each sample (interaction substrate * sample)

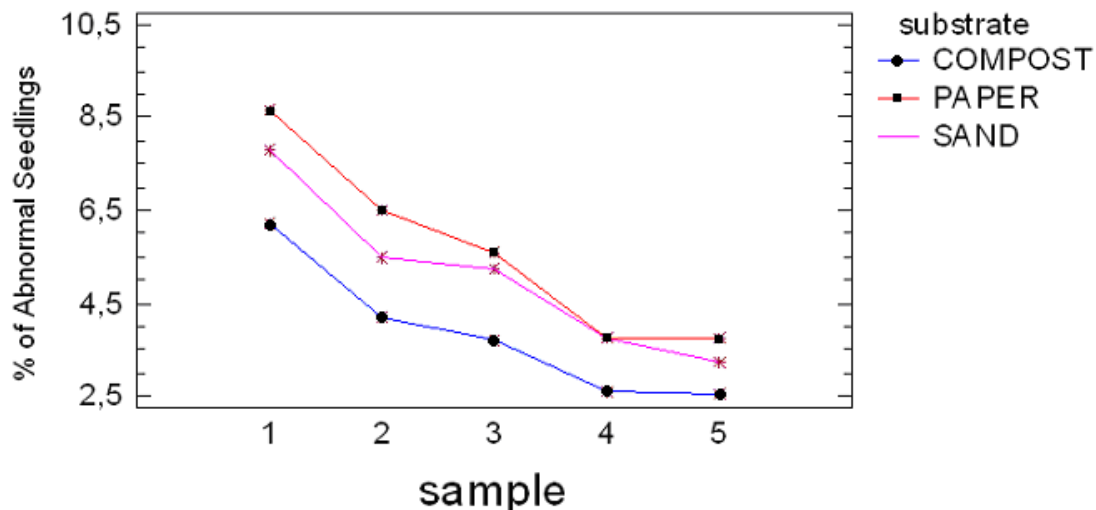


Figure 26 : % of abnormal seedlings for each sample and for each substrate.

When the results from individual samples are examined it is found that in all cases the level of abnormal is lowest in compost. Generally the highest levels of abnormal seedlings is found in paper tests. The only exception to this is sample 4 where paper and sand tests produce the same numbers of abnormal seedlings.

3.4. Comparison of substrates, for each laboratory (interaction substrate * lab)

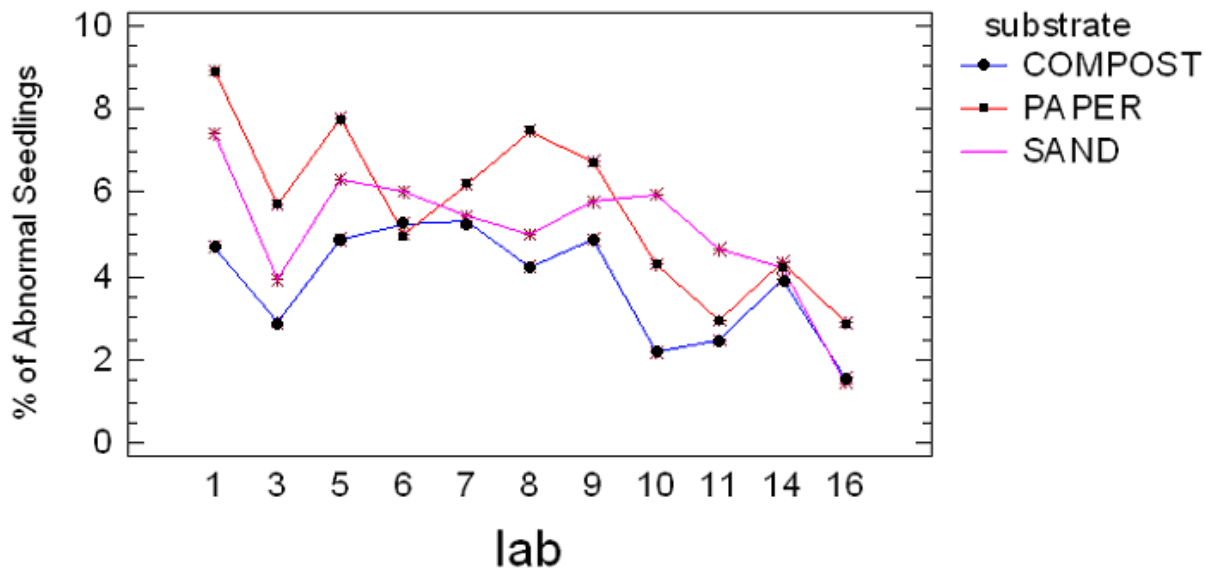


Figure 27 : % of abnormal seedlings for each laboratory and for each substrate.

When considering the results from individual laboratories, (Figure 27) it was found that the percentage of abnormal seedlings is lowest for all laboratories, apart from laboratory 6, when compost is used as the germination media. On the other hand, with the exception of laboratories 6, 10 and 11, the use of paper media results in the highest levels of abnormal seedlings.

4. Root defects

In order to understand the origin of the differences in the percentage of abnormal seedlings obtained with the 3 substrates, the data recorded by the laboratories for the different types of abnormal seedlings were analysed. Two categories of abnormal seedlings give differences for the 3 substrates : “root defects” and “seedling as a whole”.

Root defects could not be analysed in detail (i.e. Root Defect A- Primary root defective with sufficient secondary roots; and Root Defect B- Primary root defective without sufficient secondary roots) because all the laboratories did not record the detailed data.

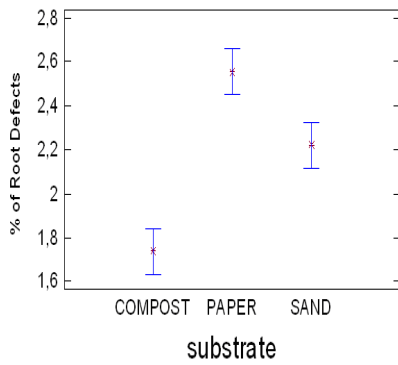


Figure 28: Mean and 95% intervals for % of root defects, for all samples, for each substrate.

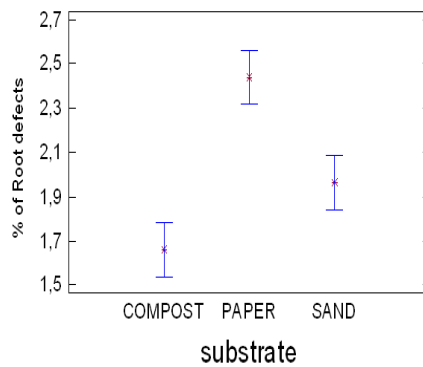


Figure 29: Mean and 95% intervals for % of root defects, for all samples, for each substrate, for ISTA laboratories .

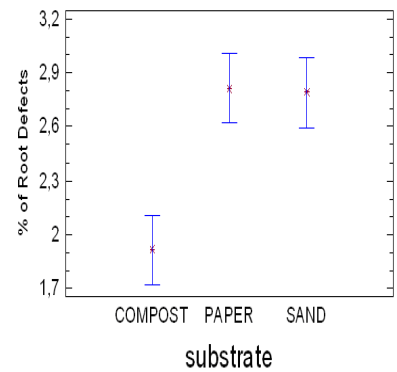


Figure 30: Mean and 95% intervals for % of root defects, for all samples, for each substrate, for AOSA laboratories .

Figure 28 demonstrates that compost leads to less root defects overall for all the laboratories whether they be ISTA or AOSA. For ISTA laboratories, paper results in more root defects and sand is intermediate between compost and paper (Figure 29). For AOSA laboratories, root defects are at similar level with paper and sand substrates (Figure 30).

AOSA laboratories find similar levels of root defects as ISTA laboratories in compost and paper substrate, but find more root defects than ISTA laboratories in sand substrate. This is an unexpected result as the primary root is considered as essential when evaluating a seedling of Sunflower as normal using ISTA rules. The primary root is not considered essential in AOSA rules provided sufficient secondary roots have developed. This is the most likely reason for ISTA laboratories identifying more root abnormalities than laboratories using AOSA methods. Sand substrate seems to increase discrepancies between ISTA and AOSA regarding the amount of root defects.

5. Defects of seedlings as a whole

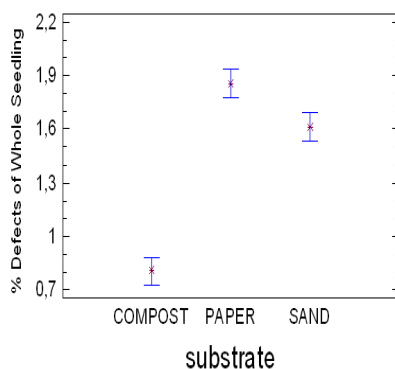


Figure 31: Mean and 95% intervals for % seedling as a whole, for all samples, for each substrate.

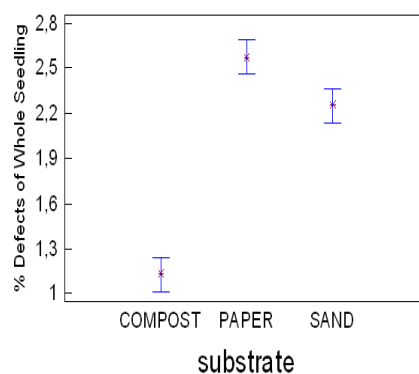


Figure 32: Mean and 95% intervals for % of seedling as a whole, for all samples, for each substrate, for ISTA laboratories.

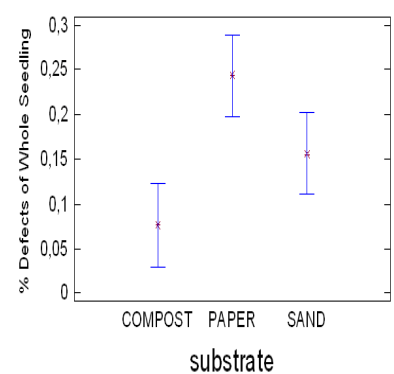


Figure 33: Mean and 95% intervals for % of seedling as a whole, for all samples, for each substrate, for AOSA laboratories.



Defects of “seedling as the whole” are defects that affect the whole seedling and not just one organ of the seedling. In this case, more than one organ of the seedling (root and shoot for example) are generally affected.

Figure 31 demonstrates that compost leads to less whole seedling defects for all the laboratories whether they be ISTA or AOSA. However, the level of whole seedling defects is very much lower for AOSA laboratories than for ISTA laboratories (Figures 32 and 33). For ISTA and AOSA laboratories, paper results in more whole seedling defects but for AOSA laboratories, the difference between whole seedling defects in paper and in sand is not significant.

Discussion - Conclusion

The objective of this comparative test and validation study was to decide whether the compost can be proposed to be included as a primary substrate in the Rules for the germination of Sunflower seeds.

To aid this decision the principal conclusions of this study are listed below:

➤ **Repeatability of the germination test using compost media**

Results obtained in this study show that sunflower germination tests conducted using compost are more repeatable than those conducted using paper and that paper tests are more repeatable than tests conducted using sand as the germination medium. This conclusion is based on an analysis of normal seedlings. Similar results are obtained when the levels of abnormal seedlings and non germinated seeds are analysed (see results in Annexe B).

➤ **Reproducibility of the germination test using compost media**

Results obtained in this study show that sunflower germination tests conducted using compost are more reproducible than those conducted using paper and that paper tests are more reproducible than tests conducted using sand as the germination medium. This conclusion is based on an analysis of normal seedlings. Similar results are obtained when the levels of abnormal seedlings and non germinated seeds are analysed (see results in Annexe C).

Composts with widely differing compositions (as it was the case in this comparative test – see page 7 and Annexe A) gave more repeatable and reproducible results than sand or paper tests. To many, this may seem quite remarkable considering the variability of the composition of the different composts used by participating laboratories. However, it should also be noted that there were considerable variations in the compositions and water content of the sand and paper media used by the participating laboratories.



➤ **Comparison of the germination results obtained using compost with the results obtained using prescribed methods (paper and sand)**

- ***Normal seedlings***

Germination with compost gives more normal seedlings than with paper and paper gives more normal seedlings than sand

- **Abnormal seedlings**

Germination with compost gives less abnormal seedlings than with sand and sand gives less abnormal seedlings than in paper. Using paper substrates, there are more root defects and more defects of the whole seedling.

- **Ungerminated seeds**

Germination with compost reduces the number of ungerminated seeds compared to paper (significant for ISTA laboratories, not significant for AOSA laboratories). Germination with sand results in more ungerminated seeds than paper or compost.

In conclusion, germination of Sunflower seeds is optimal in compost substrate, whatever the type of compost used by the laboratories. This is not the case for the germination with the 2 other substrates: paper that gives more abnormal and sand that gives more ungerminated seeds.

Compost can therefore be proposed to be included in ISTA Rules as a primary medium for germination of Sunflower seeds – in addition to sand and paper that are already considered primary germination medias for Sunflower germination. Other conditions (especially temperature) prescribed for germination test of Sunflower should remain the same as before, as they have been already selected to give optimal germination results for this species.

Compost is not a new substrate for laboratories testing germination. It is already used by laboratories for retests when germination is affected by chemical seed treatments which have caused phytotoxic abnormal seedlings in paper and sand tests. This proposition of including compost as a primary substrate for Sunflower germination test will be associated with a proposition to modify the paragraph dealing with substrates in the ISTA Rules.



Annexes

Summary

Annexe A – Tables of the methods used by the laboratories

Annexe B – Analysis of repeatability for abnormal and ungerminated seeds

Annexe C – Analysis of reproducibility abnormal and ungerminated seeds

Annexe D – Analysis of variance - For all the laboratories
(including temperatures differing from 20°C)

Annexe E – All the data of normal, abnormal, fresh and dead seeds
– File Excel “Annexe E “

Annexe F – All the detailed data of abnormal seedlings
– File excel “Annexe F“



ANNEXES A

Tables of the methods used by the laboratories

➤ **Table 1 : Methods of germination used by the laboratories with SAND substrate**

LABORATORY	SUBSTRATE 1 : composition 2 : number of layers of paper per box 3 : quantity of sand or compost per box	NUMBER OF SEEDS per box or rolled towel	SIZE of the box and paper layers (cm)	AMOUNT OF WATER ADDED (1): % of volume of the dry substrate (2): % of weight of the dry substrate	TEMPERATURE (°C)	LIGHT (hours per day)	INTERMEDIATE COUNTS AND FINAL COUNT (days)
LAB 1	1 :Silicate sand from quarry mesh size in mm (0.425 – 0.075) 2 : 3 : 1100 g per box	50	(18 x 12 x 5.5)	(1) : 9%	20	8	7
LAB 2	1 : Sand / quartzsand Water capacity : 28.6% 2 : 2 3 : 2 450 g	50	(30 x 25 x 6)	(1) : 5.38% (2) : 9.88%	25	10	10
LAB 3	1 : Quartz sand (0.3-0.9 mm) 2 : 1 layer paper 3 : 1 632.5 g	8 x 50	(17 x 12.5 x 6)	(2) : 9.7%	20	12	7
LAB 4	1 : Sand from quarry 2 : 3 : about 1 Liter	8 x 50	(18 x 12 x 5.5)	(1) : 10%	20-30	16	7
LAB 5	1 : River sand (0.05 < 93% < 0.8) mm 2 : 3 : 1 Liter	50	(18 x 12 x 5.5)	(1) : 20% (2) : 12%	20	8	7
LAB 6	1 : 0.4 – 1.0 mm 2 : 3 : 0.5 Liter + 0.25 Liter to cover (2 cm + 1 cm to cover)	50	(12.5 x 18.5)	(1) : 5 sand + 1 water = 17%	20	8	10
LAB 7	1 : 2 : 3 : 750 – 1000 g	50	(17 x 14 x 4.5)	Sand 10 : water 1 dry sand (9%)	20	8	7, 10
LAB 8	1 : Silvapearl, silver sand washed and graded 2 : 3 : 5 703 g	50	(33.5 x 21.5 x 5.5)	(1) : 30% (2) : 19%	20	12	10, 12

LAB 9	1 : Fine white, Garside grade 60 0.05-0.8 mm 2 : 3 : about 2 Kg per seed tray	50	Each seed tray =(22 x 16.5 x 6)		20	24	7, 10
LAB 10	1 : Small uniform particles < 0.8 mm pH 6.5 2 : 3 : 2 500 g	50	(24 x 19 x 4)	(1) : 32% (2) : 19.3%	20	8	7, 10
LAB 11	1 : 0.05 – 0.8 mm mixed quartz aggregate 2 : 3 : 280 ml	100	(38 x 25)	(1) : 21.1% (2) : 15.1%	20	8	7
LAB 12	1 : Silica sand – mesh size 1 mm (24 mesh) 2 : 3 : approximately 500 ml	50	(11.5 x 11.5 x 4)	(1) 12.5% (2) 8.7%	25	8	7
LAB 14	1 : Silica 2 : 1 blotter on box bottom 3 : 600 g	100	(15.2 x 22.9)	(2) : 6%	20	10	9
LAB 16	1 : kiln dried sand 2 : 3 : 3 – 2 L cans of sand	4 x 100	(47.5 x 66)	(1) : 23 to 35%	20	12	8

*: For sand (type of sand and size), for compost (% of peat and % of sand or other constituent)

➤ **Table 2 : Methods of germination used by the laboratories with PAPER substrate**

	SUBSTRATE 1 : composition 2 : number of layers of paper per box 3 : quantity of sand or compost per box	NUMBER OF SEEDS per box or rolled towel	SIZE of the box and paper layers (cm)	AMOUNT OF WATER ADDED (1): % of volume of the dry substrate (2): % of weight of the dry substrate	TEMPERATURE (°C)	LIGHT (hours per day)	INTERMEDIATE COUNTS AND FINAL COUNT (days)
LAB 1	1 : Pleated paper 2 : 1 sheet of the flat paper and 1 sheet of the 50 pleats 3 :	50	(18 x 12 x 5.5)	(2) : 176%	20	8	7
LAB 2	1 : Pleated paper Water capacity : 251% 2 : 3 : 2	50	BOX : (18 x 13.5 x 6.5) PAPER : (11 x 2)	40 ml	25	0	4, 10
LAB 3	1 : 2 : 3 layers per towel (2 at bottom, 1 on top) 3 :	8 x 50	(62 x 14.5)	Saturated	3 days at 10 7 days at 20	No 12	10
LAB 4							
LAB 5	1 : ANCHOR paper 2 : 2 + 1 layer Wax paper 3 :	50	(38 x 25.4)	Saturated	20	No	7
LAB 6	1 : 2 : 3 : 2 under, 1 cover (roll)	50	(20 x 58)	(2) : 70%	2 days for prechill 20	8	7
LAB 7	1 : 2 : 3 3 :	50	(42.5 x 24.5)	3 p dry 27 g 3 p wet 82 g (2) : 303 %	20	8	7, 10
LAB 8	1 : Rolled paper towel 2 : 3 on bottom + 3 on top 3 :	50	(38 x 23 x 1)	(1) : 75% (2) : 82%	20	12	7, 10

LAB 9	1 : Rolled towel 2 : 2 underneath seed, 2 covering seed	50	(37 x 23)		20	24	7, 10 (14 days for 6 samples)
LAB 10	1 : Wood, Ph 7.0, porous 2 : 3 3 :	50	(45.1 x 215)/ layer	16 ml per layer	20	8	7, 10
LAB 11	1 : Rolled towel 2 : 2 3 :	50/towel	(38 x 25)	(2) : 352%	20	8	7
LAB 12	1 : Rolled towel, regular weight seed germination paper (38 lbs) 2 : 3 layers 3 :	50	(10 x 15)	50 ml water added to 3 sheets of rolled paper	25	8	7
LAB 14	1 : 2 : 2 3 :	100	(38.1 x 50.8)	(2) 225%	20	10	8
LAB 16	1 : Rollers towels 2 : 2 layers (1 on top and 1 on bottom) 3 :	100 seeds per 2 towels	(61 x 30)	Wet thoroughly then pressed	20	12	7, 8

*: For sand (type of sand and size), for compost (% of peat and % of sand or other constituent)

➤ **Table 3 : Methods of germination used by the laboratories with COMPOST substrate**

	SUBSTRATE 1 : composition 2 : number of layers of paper per box 3 : quantity of sand or compost per box	NUMBER OF SEEDS per box or rolled towel	SIZE of the box and paper layers (cm)	AMOUNT OF WATER ADDED (1): % of volume of the dry substrate (2): % of weight of the dry substrate	TEMPERATURE (°C)	LIGHT (hours per day)	INTERMEDIATE COUNTS AND FINAL COUNT (days)
LAB 1	1 : 80% of peat and 20% of river sand 2 : 3 : around 600 g per box	50	(18 x 12 x 5.5)	(1) 35-40 %	20	8	7
LAB 2	1 : 100 % Baltic up land peat Ph : 5.5-6.5 Content of salt < 1.5 g/l ▪ N 60-150 mg/l ▪ P ₂ O ₅ 50-150 mg/l ▪ K ₂ O 80-200 mg/l 2 : None 3 : None	50	(30 x 25 x 6)		25	10	10
LAB 3	1 : 80 % peat, 20 % sand 2 : 1 layer paper 3 : 1 Liter	8 x 50	(17 x 12.5 x 6)	(1) : 2%	20	12	7
LAB 4	1 : Mix of peat (80%) and sand (20%) 2 : 3 : About 1 Liter	8 x 50		(2) : 45 to 50 %	20-30	16	7
LAB 5	1 : 80 % compost + 20 % river sand 2 : 3 : 1 Liter	50	(18 x 12 x 5.5)	(1) 2 % (2) 3 %	20	8	7
LAB 6	1 : 33 % peat, 67 % artificial soil 2 : 3 : : 0.5 Liter + 0.25 Liter to cover (2 cm + 1 cm to cover)	50	(12.5 x 18.5)	By organ sense, the soil is pre-moistened by the manufacturer	20	8	11

LAB 7	1 : 80 % peat, 20 % sand 2 : 3 : 500 g	50	(17 x 14 x 4.5)	Compost 7 : water 1 = 12.5%	20	8	7, 10
LAB 8	1 : F2 Levington with sand Peat / sand/ nutrients, pH = 5.3-6.0 N 5, P 8, K 3 and Mg 7 2 : 3 : 1 776.4 g	50	(33.5 x 21.5 x 5.5)	(1) : 32 % (2) : 146 %	20	12	10 (or 8)
LAB 9	1 : 95% peat, 5% sand N 192 g.m ⁻³ , P ₂ O ₆ 224 g.m ⁻³ K ₂ O 384 g.m ⁻³ 2 : 3 : about 700 g per seed tray	50	Each seed tray =(22 x 16.5 x 6)		20	24	7,10
LAB 10	1 : Ph 7.3 N 2%, P 0.68%, K 0.94%, Organic Matter 34.76% and Moisture 40.2% 2 : 3 : 666 g	50	(24 x 19 x 4)	(2) : 40 %	20	8	12, 14
LAB 11	1 : 20% perlite/ 80% peat 2 : 3 : 280 ml	100	(76 x 25.5)	(1) : 26.3% (2) : 76.1%	20	8	7
LAB 12	1 : Soil, 82 % compost, 18% silica sand 2 : 3 : approximately 300 ml	50	(11.5 x 11.5 x 4)	Add water until sand can be formed into a ball when squeezed into the palm of the hand, the ball breaking freely when pressed between two fingers.	25	8	7
LAB 14	1 : 75% peat moss + perlite, dolomitic limestone, gypsum & wetting agent 2 : 1 sheet paper base on tray 3 : 150 g dry weight per 100 seeds	100	(23 x 30) with 2 reps on (48 x 51) tray	(2) : 300%	20	10	9
LAB 16	1 : Sphagnum peat, perlite, wetting agent and fertilizer 2 : 3 : 3 – 2 L cans of compost	4 x 100	(47.5 x 66)	(1) : 23 to 35%	20	12	8

*: For sand (type of sand and size), for compost (% of peat and % of sand or other constituent)



ANNEXES B

Analysis of repeatability

Annexe B.1 : Analysis of repeatability towards germination quality,
For abnormal seedlings and ungerminated seeds

Annexe B.2 : Analysis of repeatability by sample for all substrates,
For abnormal seedlings and ungerminated seeds

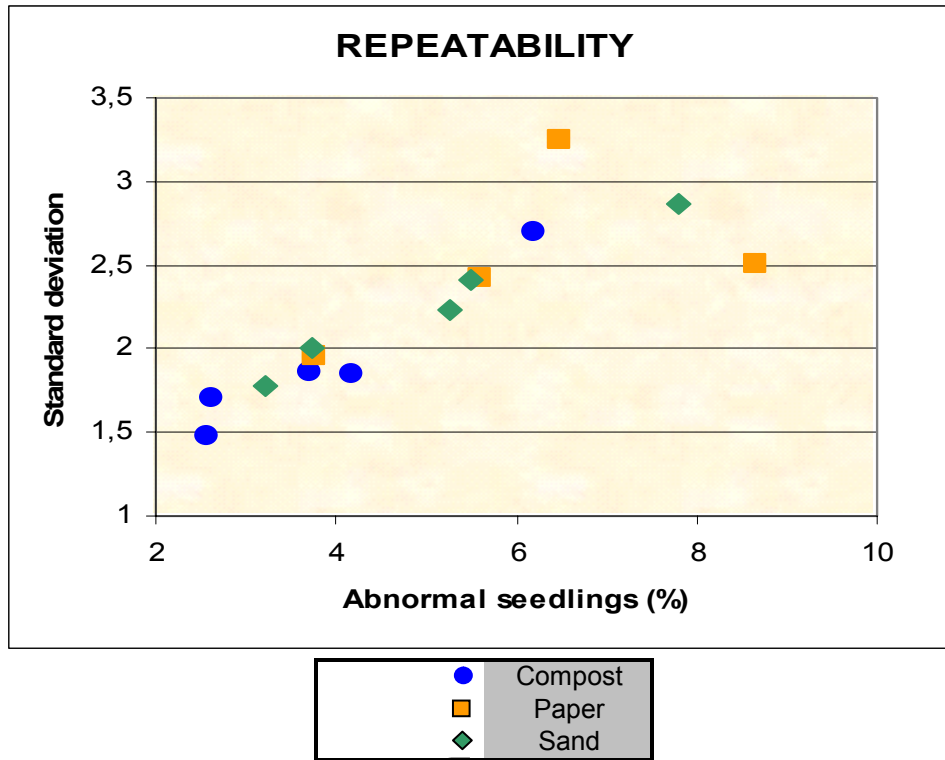


Figure B.1.1. : Variation of repeatability towards germination quality (% of abnormal seedlings) of the samples for all the substrates tested.

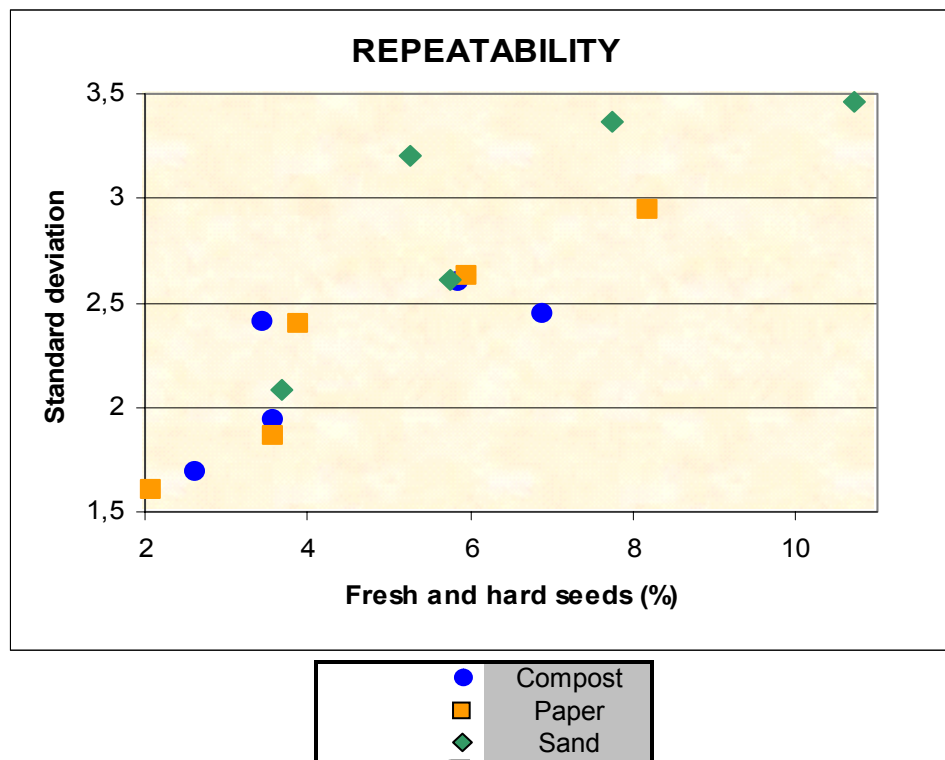


Figure B.1.2. : Variation of repeatability towards germination quality (% of ungerminated seeds) of the samples for all the substrates tested.

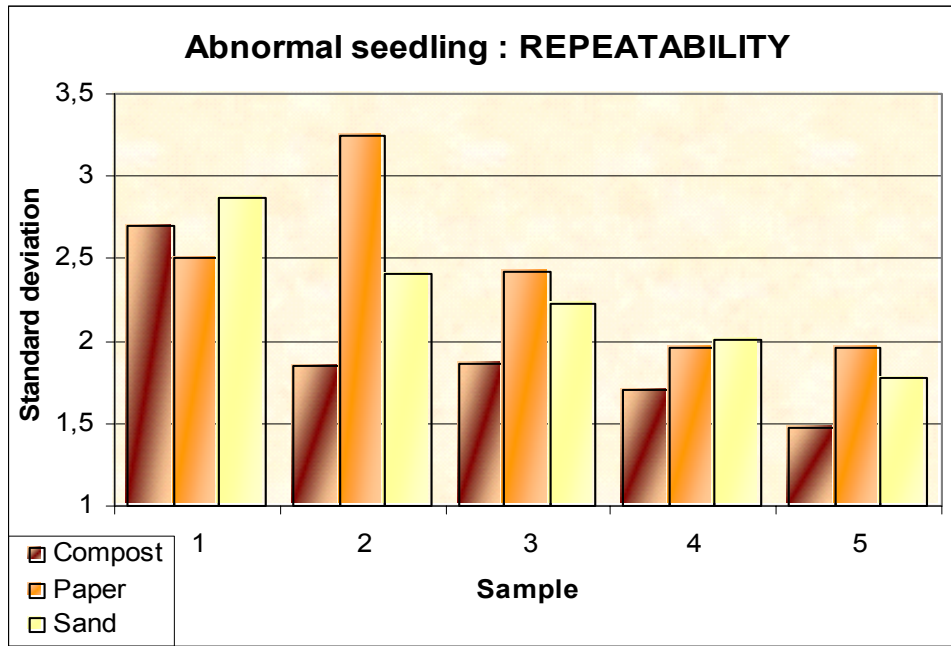


Figure B.2.1. : results of repeatability by sample and by substrate for the percentage of abnormal seedlings.

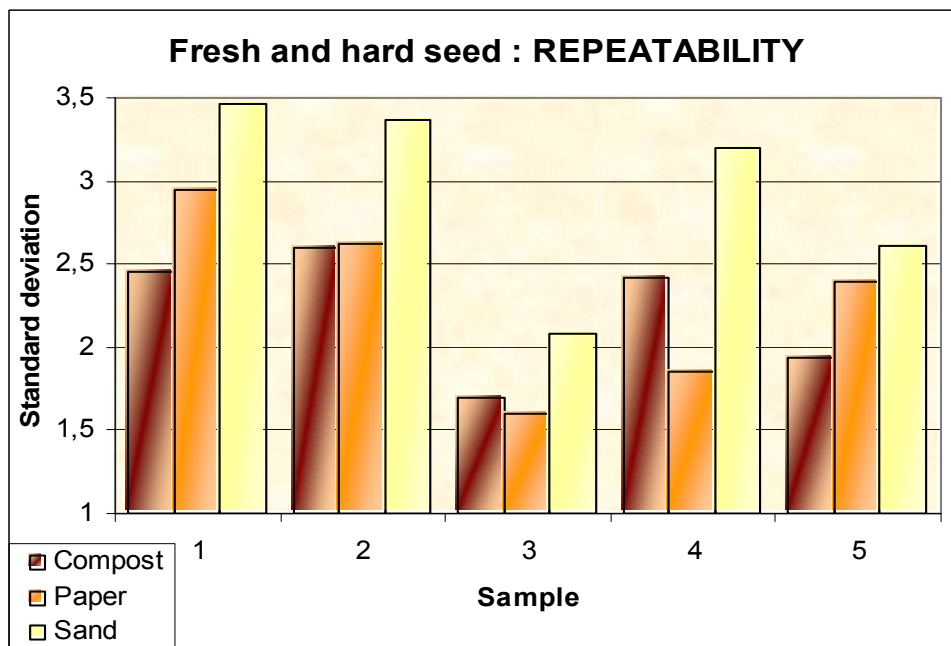


Figure B.2.2. : results of repeatability by sample and by substrate for the percentage of ungerminated seeds.



ANNEXES C

Analysis of reproducibility

Annexe C.1 : Analysis of reproducibility towards germination quality,
For abnormal seedlings and ungerminated seeds

Annexe C.2 : Analysis of reproducibility by sample for all substrates,
For abnormal seedlings and ungerminated seeds

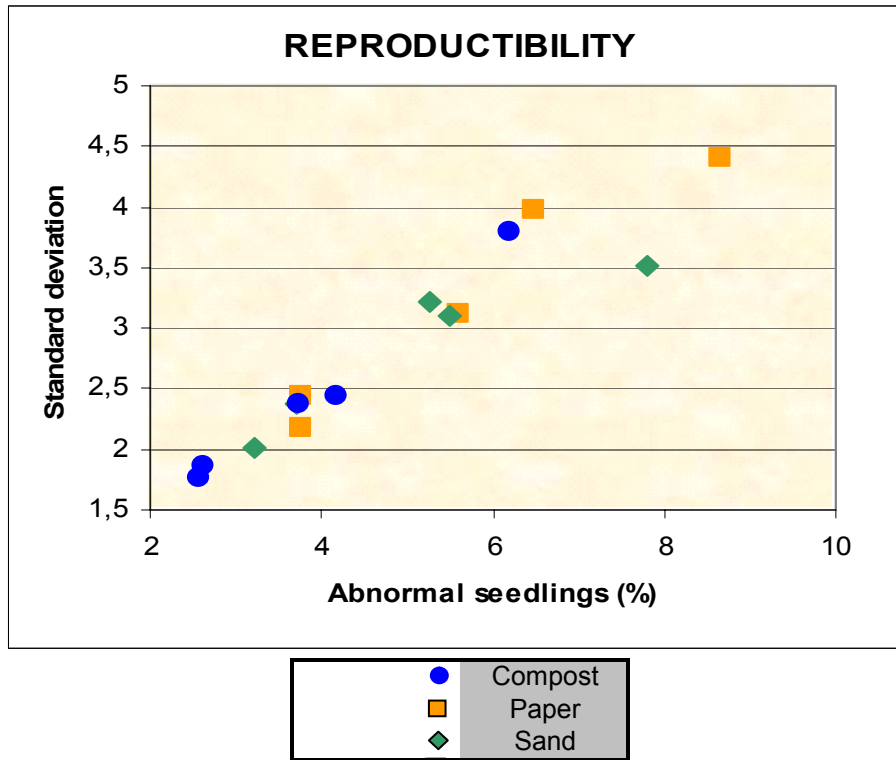


Figure C.1.1. : Variation of reproducibility towards germination quality (% of abnormal seedlings) of the samples for all the substrates tested.

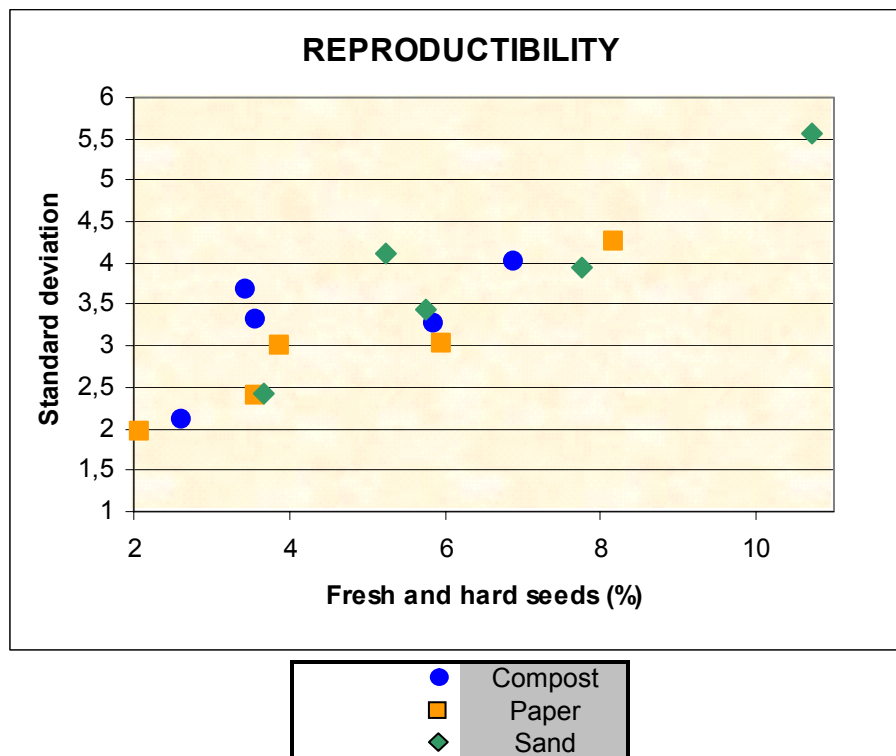


Figure C.1.2. : Variation of reproducibility towards germination quality (% of ungerminated seeds) of the samples for all the substrates tested.

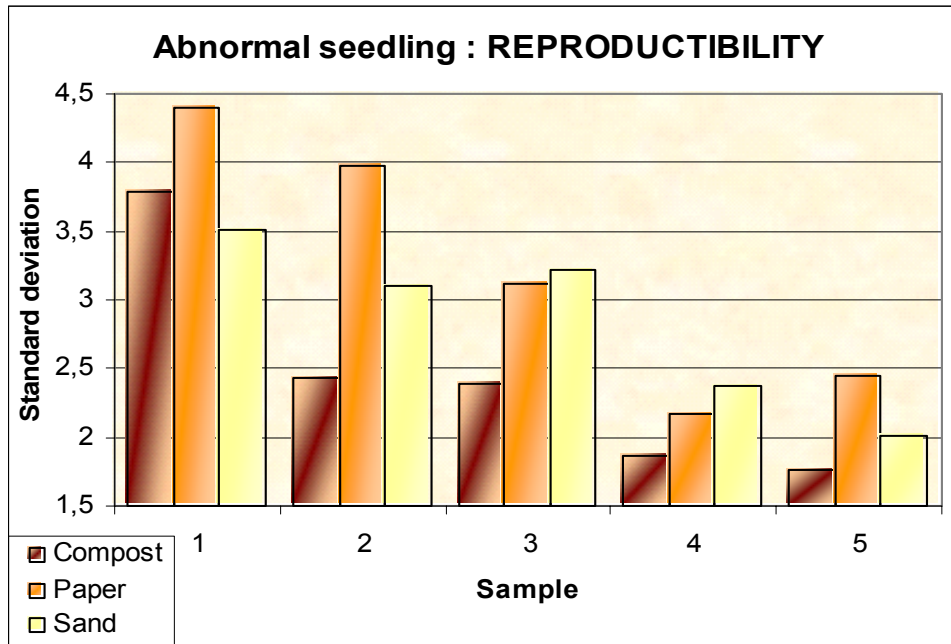


Figure C.2.1. : results of reproducibility by sample and by substrate for the percentage of abnormal seedlings.

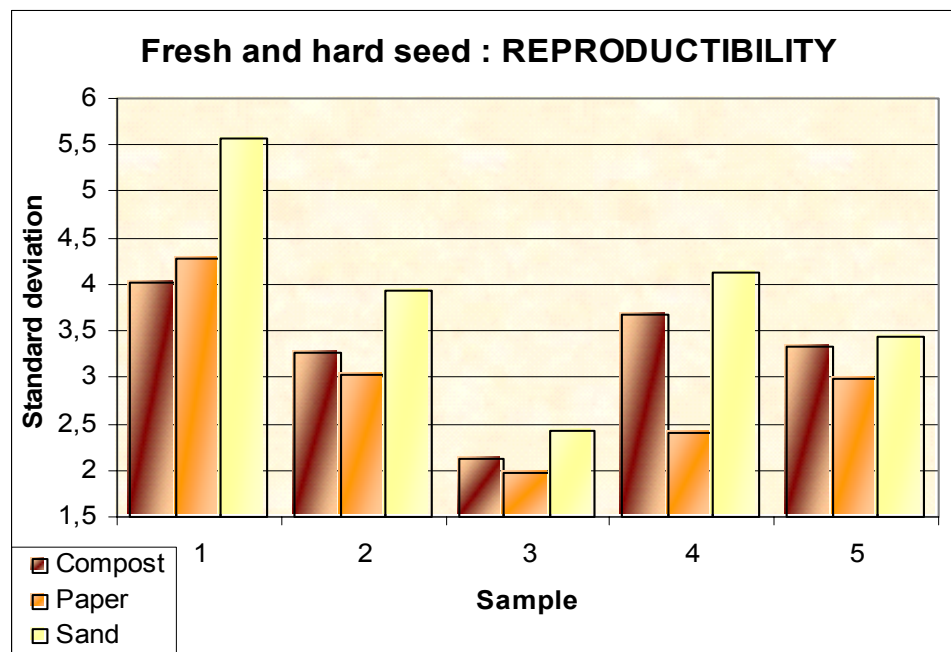


Figure C.2.2. : results of reproducibility by sample and by substrate for the percentage of ungerminated seeds.



ANNEXES D

Analysis of Variance

For all the laboratories (including temperatures differing from 20°C)

Annex D.1. : Normal Seedlings

- Comparison of substrates, all samples together
- Comparison of substrates, for each sample (interaction substrate * sample)
- Comparison of substrates, for each laboratory (interaction substrate*lab)
- Comparison of substrates, for ISTA and AOSA laboratories

Annex D.2 : Ungerminated Seeds (Fresh + Dead seeds)

- Comparison of substrates, all samples together
- Comparison of substrates, for each sample (interaction substrate * sample)
- Comparison of substrates, for each laboratory (interaction substrate*lab)
- Comparison of substrates, for ISTA and AOSA laboratories

Annex D.3.: Abnormal Seedlings

- Comparison of substrates, all samples together
- Comparison of substrates, for each sample (interaction substrate * sample)
- Comparison of substrates, for each laboratory (interaction substrate*lab)
- Comparison of substrates, for ISTA and AOSA laboratories



Normal Seedlings

D.1.1. Comparison of substrates, all samples together

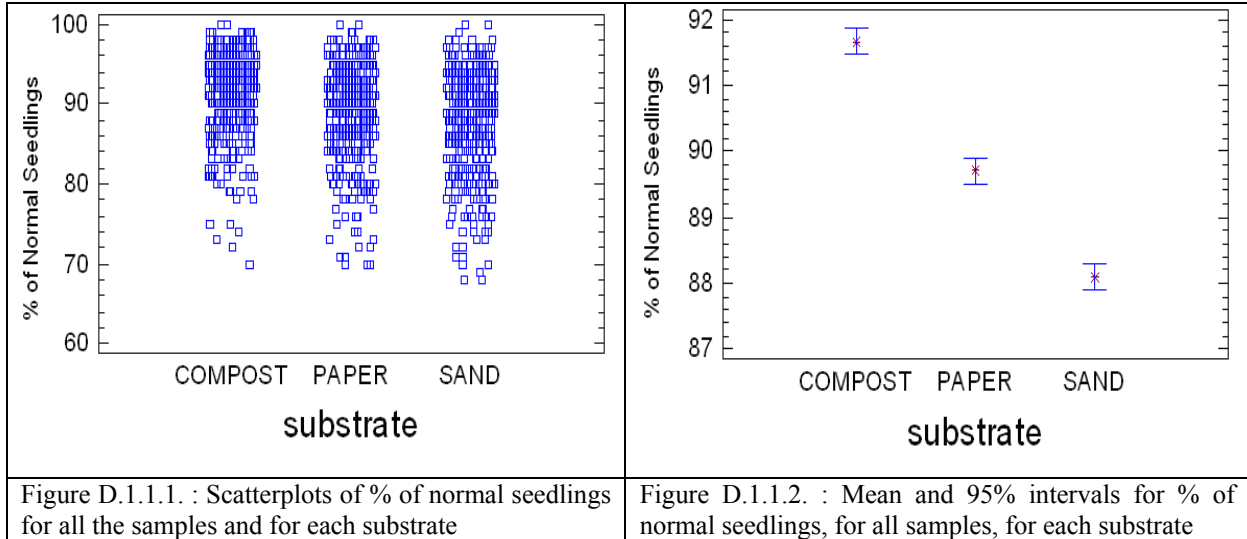


Figure D.1.1.1. : Scatterplots of % of normal seedlings for all the samples and for each substrate

Figure D.1.1.2. : Mean and 95% intervals for % of normal seedlings, for all samples, for each substrate

D.1.2. Comparison of substrates, for each sample (interaction substrate * sample)

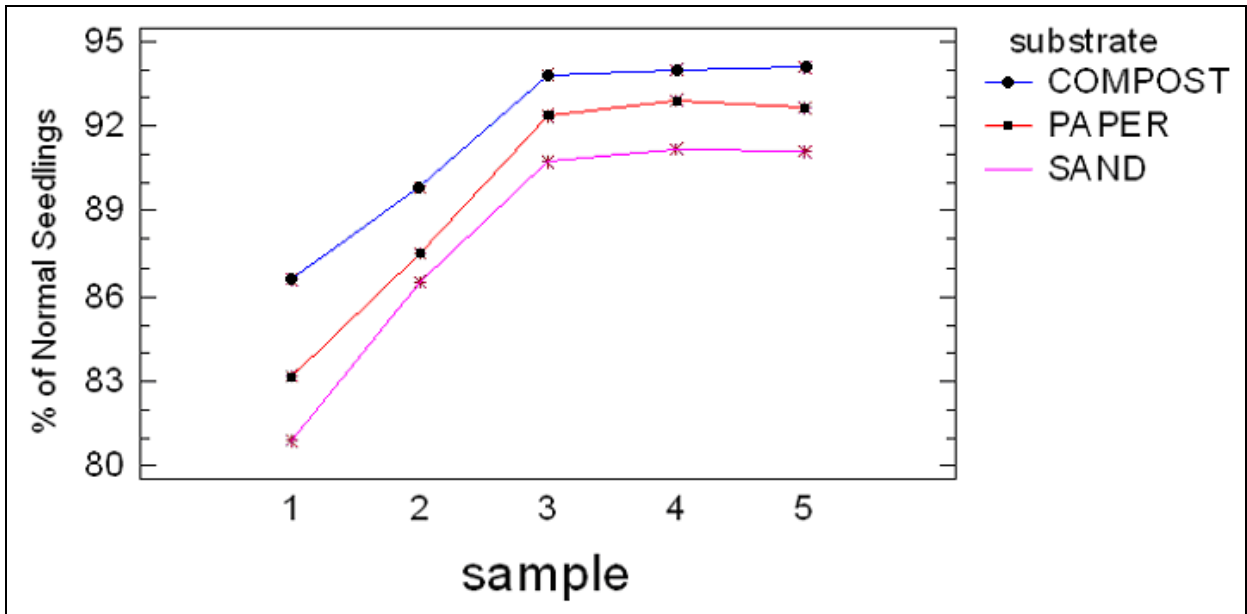


Figure D.1.2.1. : % of normal seedlings for each sample and for each substrate.



*D.1.3. Comparison of substrates, for each laboratory (interaction substrate * lab)*

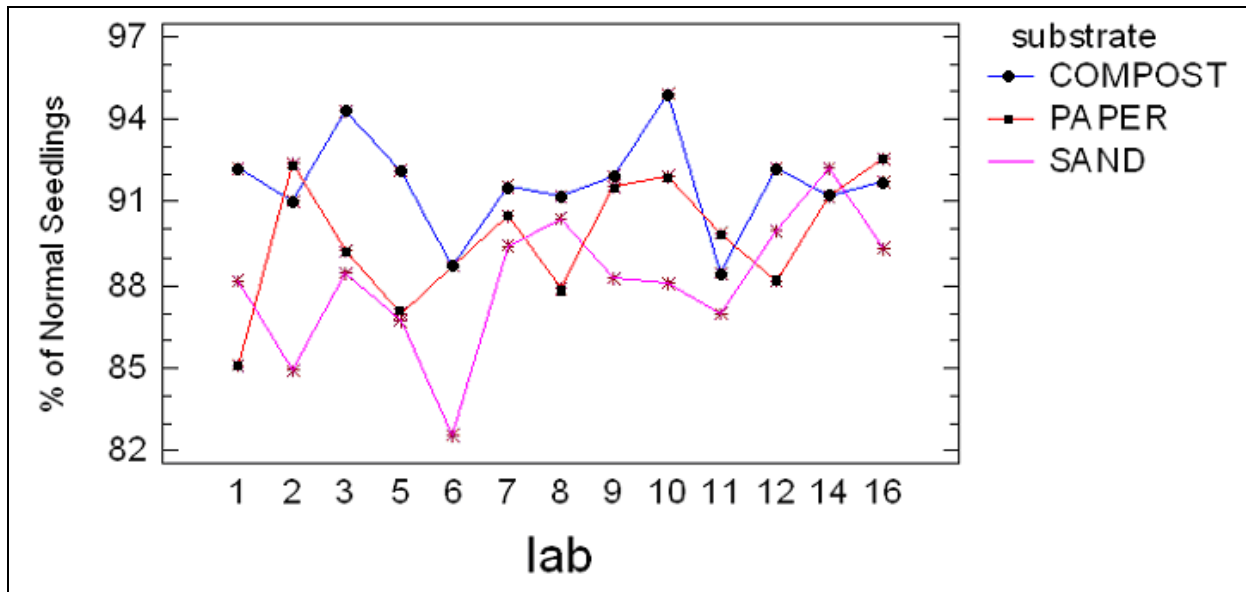
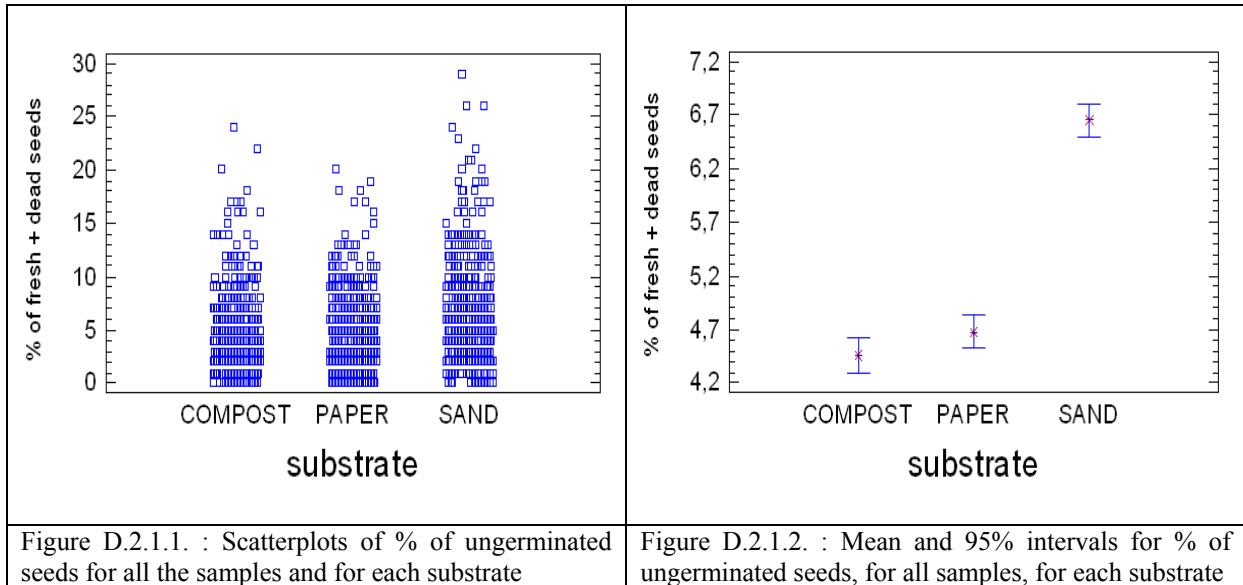


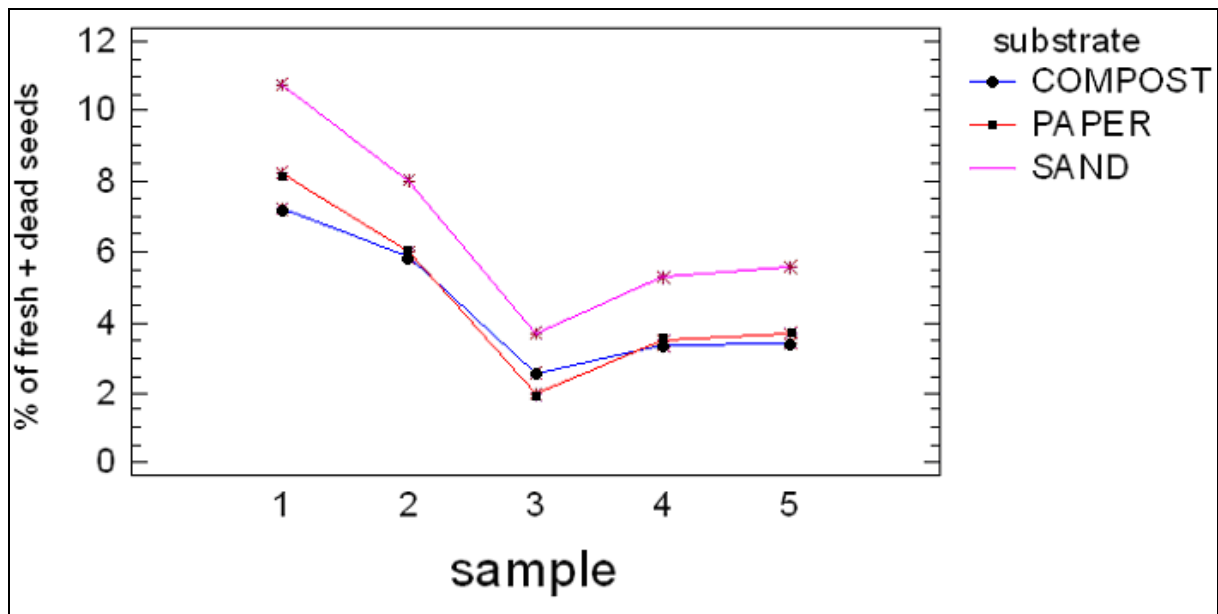
Figure D.1.3.1.: % of normal seedlings for each laboratory and for each substrate.

Ungerminated Seeds (Fresh + Dead)

D.2.1. Comparison of substrates, all samples together



D.2.2. Comparison of substrates, for each sample (interaction substrate * sample)





*D.2.3. Comparison of substrates, for each laboratory (interaction substrate * lab)*

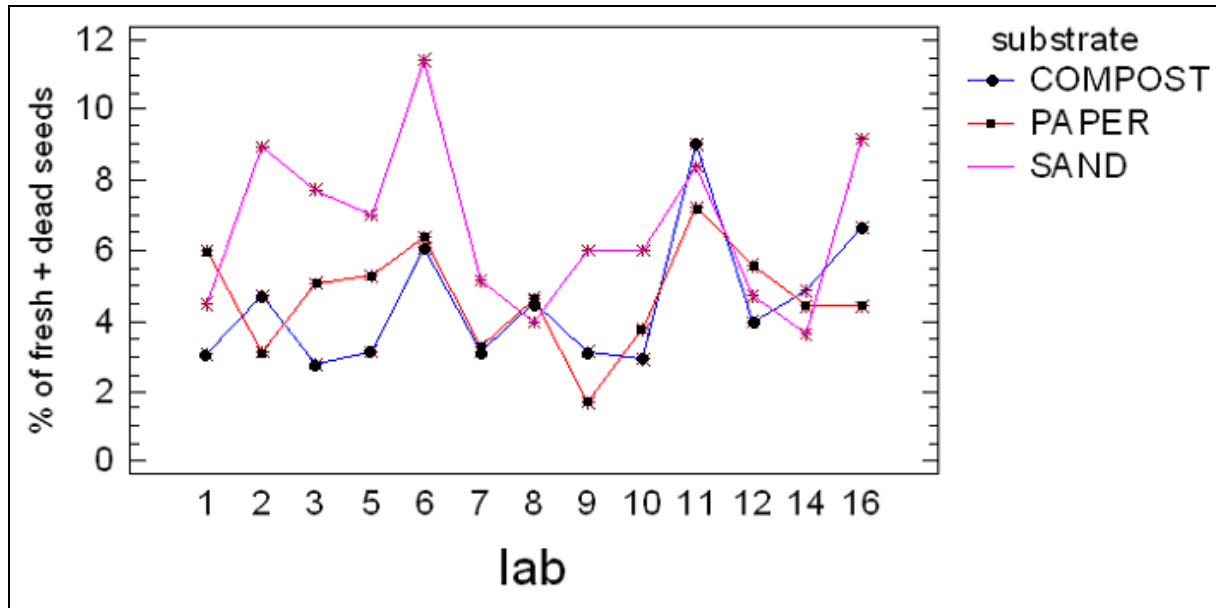


Figure D.2.3.1.: % of ungerminated seeds for each laboratory and for each substrate.

Abnormal Seedlings

D.3.1. Comparison of substrates, all samples together

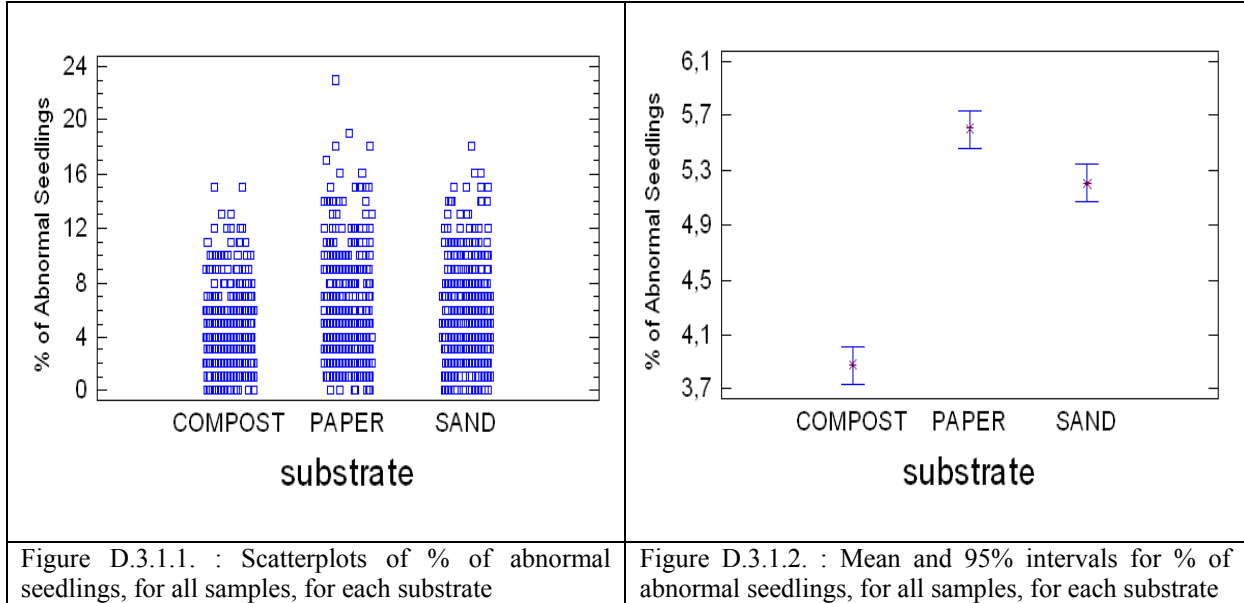


Figure D.3.1.1. : Scatterplots of % of abnormal seedlings, for all samples, for each substrate

Figure D.3.1.2. : Mean and 95% intervals for % of abnormal seedlings, for all samples, for each substrate

D.3.2. Comparison of substrates, for each sample (interaction substrate * sample)

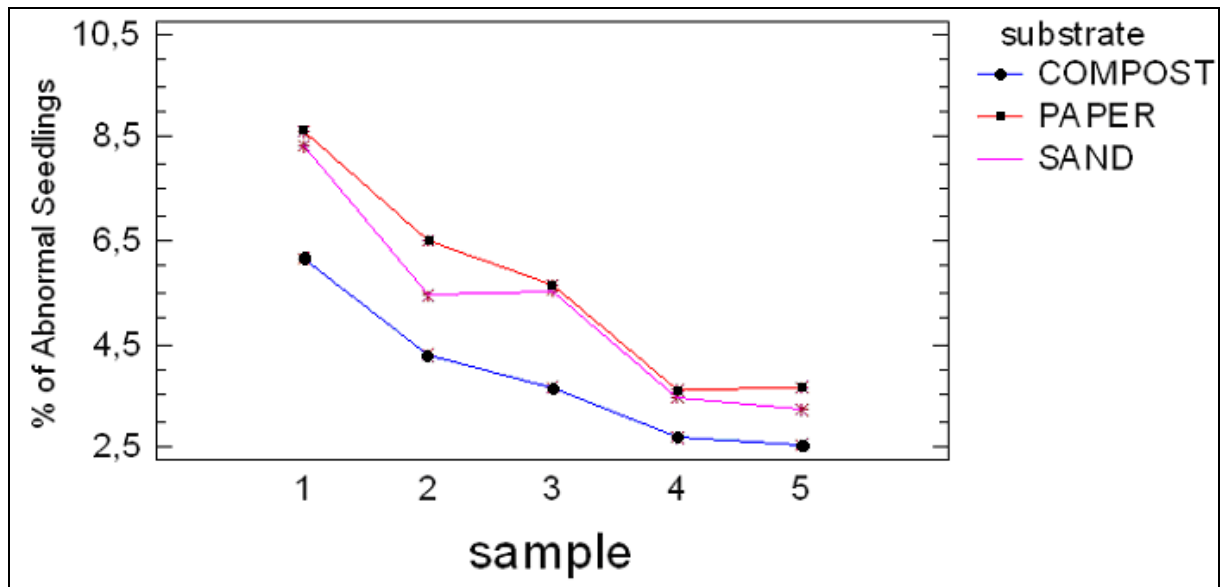


Figure D.3.2.1. : % of abnormal seedlings for each sample and for each substrate.



*D.3.3. Comparison of substrates, for each laboratory (interaction substrate * lab)*

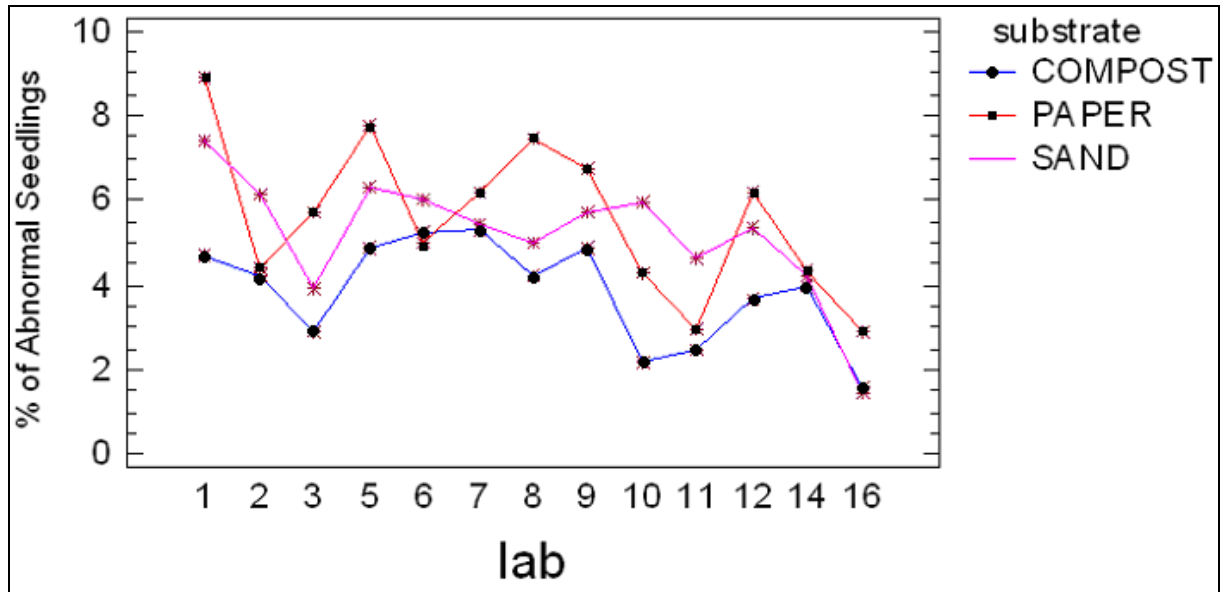


Figure D.3.3.1.: % of abnormal seedlings for each laboratory and for each substrate.