THE USE OF THE TETRAZOLIUM TEST AS A MEASURE OF GERMINATION VIGOR AND CHEMICAL TOXICITY IN SEEDS

by

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ABSTRACT

Experiments were conducted during the years 1964 and 1965 in the Seed Technology Laboratory of the American University of Beirut to study the use of the tetrazolium test in estimating seed viability and seedling vigor. The tetrazolium test was also used to detect the chemical toxicity of mercuric chloride on barley seed and of Panogen-15 on corn, wheat, barley, sorghum, cotton, and vetch.

The results reveal that a good estimation of viability was obtained by the tetrazolium test in corn, barley, wheat, sorghum, cotton, and vetch, in that most of the samples when tested by tetrazolium chloride gave comparable results with the germination test.

Evaluation of vigor was possible by the tetrazolium test in corn, sorghum, barley and cotton. This test failed to evaluate vigor in vetch seed. The deeply stained seed of corn, barley and sorghum were considered vigorous and the light stained as less vigorous. No difference in the intensity of coloration was observed in cotton between vigorous and less vigorous seed, instead necrosis of the radicle and cotyledonary tissue appeared to be responsible for low vigor. Neither necrosis of the

embryonic tissue nor the intensity of coloration could be used in the evaluation of vigor in vetch seed.

Chemical toxicity caused by mercuric chloride in barley seed and by Panogen-15 in corn and cotton was not detected by the tetrazolium test. Toxicity caused by Panogen-15 in barley, wheat, sorghum, and vetch was in fair agreement with laboratory germination test results.

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INTRODUCTION

It is an established and well known fact that a seed lot is of value only if a high percentage of the seed is viable. The need to know the viability of the seed is of utmost importance to the buyer as well as to the seller. According to the rules of the International Seed Testing Association (1) the time required for germination varies between seven to fourteen days for most crop seeds and may go up to ninety days for tree seeds.

The long periods of time required for the completion of germination tests have hindered progress towards greater efficiency in seed plant and marketing operations. Decisions regarding processing procedures, bulking, blending and disposing the seed lots are usually delayed until the results of the germination tests are available. It is worthwhile to mention that other factors for seed quality, such as purity and amount of noxious weed seeds present in the seed can be determined within a few hours. Determination of moisture percentage in the seed sample can also be done in an even shorter period of time. Therefore, the need for determining the viability of the seed lot within a shorter period of

time is of paramount importance. Several workers have been investigating different means and methods for rapid estimation of seed viability. The use of tetrazolium salts for rapid seed viability testing has been known to seed analysts for more than two decades. There is a good number of tetrazolium salts that can be used for the determination of viability of seeds. The most commonly used one is technically known as 2, 3, 5-Triphenyl tetrazolium chloride which is a white powder and colorless in solution. On contact with the living tissue of the seed embryo the chemical is reduced by the activities of the dehydrogenase enzymes present in the seed to an insoluble and nondiffusible red formazan which stains the embryo. Since the dehydrogenase enzymes are not present in the dead tissues, dead parts in the embryo remain unstained (15). As such the tetrazolium test is not a direct test for germination but a measure for the enzymatic activity within the embryo. Fortunately enough, however, this enzymatic activity is highly correlated with the viability of the seeds and by this test the germinability of the seed can be estimated.

Among the quality factors of seed that are affected by natural or artificial ageing, are seed viability and seed vigor. Of these two important factors

of seed quality, only viability is adequately evaluated under the present seed testing procedures. The decline of vigor usually occurs at a much more rapid rate than does viability. A seed lot is of little value for planting if it has lost its vigor even if it is still highly viable. Under such conditions, therefore, the test of vigor will be a more realistic measure of the degree of seed deterioration. A good number of workers at present are testing the use of tetrazolium chloride in the evaluation of vigor.

The benefit of seed treatment and seed disinfection by different chemicals against seed-borne microorganisms has been well established for a long time. These chemicals may cause different levels of toxicity especially if high concentrations are used.

In the present study the tetrazolium test was used to evaluate viability and vigor as well as to detect chemical injury in seed.

REVIEW OF LITERATURE

The systematic and scientific search for rapid methods for estimating the germinative behaviour of seed dates back to the nineteenth century. This search for techniques took many directions, some of which have already been proved inaccurate, while others have considerable potential but have not been fully exploited (8).

According to Delouche *et al.* (8) Waller was the first to report on an electrical method for determining viability of seeds. Viable seeds when subjected to an electrical current gave so called "blaze currents" which could be measured galvanometrically, and that dead seed reacted differently to the treatment. Subsequent work on this method showed that the technique was fairly reliable but very time consuming and required considerable technical competence.

Testing for viability by an electrical method was reported by Hibbard and Miller (20). Their experiments were based upon the principle that non-viable seeds were more permeable than viable seeds and as a result electrolytes would leach out more easily from dead or non-viable seeds when the seeds were soaked in a

dilute solution of potassium permanganate. By measuring the electrical resistance it was found that resistance varied directly with viability. They concluded that the viability of a seed lot can be determined by this method with some accuracy.

Presley (36) used this electrical resistance technique in determining cotton seed viability. Permeability of the protoplast to electrolytes was found to have inverse relation to viability of the seed. The healthy protoplast allows only small quantities of electrolytes to leach through its membranes. Injury results in changes within the protoplast which alter the semi-permeable properties of its membranes. In laboratory germination he observed that fungus growth was more abundant and earlier in appearance on seeds that were progressively more deteriorated and which had relatively low resistance readings. Hence, it appeared probable that seed of low viability would actually stimulate microbial growth in the soil and thus increase seedling disease hazards, resulting in poor stands.

Lasage (28) attempted to discover a quick method that would replace the germination test. The method was based on the phenomenon of differential rates of diffusion of substances from live and dead seeds. He prepared twenty different solutions of potassium

hydroxide of increasing concentrations and soaked the seeds in them and later measured the development of color in the solutions. It was observed that non-viable seeds imparted a yellow color in all the solutions, but that only the most concentrated solutions were colored by the viable seeds. Gadd (11) reported on the work of Qvam that seeds with high viability produce more carbon dioxide per unit time than seeds with low viability. On the other hand, Delouche *et al.* (8) mentioned that it was reported by Bacquerel that dead seeds were capable of producing more carbon dioxide than viable seeds in consequence of the activity of microorganisms.

In 1914 Darsie *et al.* (7) measured the increase in temperature arising during germination and connected this with the viability of the seed. It should be mentioned here that none of the above mentioned methods gave an estimate of the germinability of individual seeds. Several workers tried to determine the viability of individual tree seeds with x-ray photography and x-ray contrast method in recent years. Olsen and Simak (35) reported that the viabilities of *Pinus silvestris* seeds could be easily and quickly determined by means of x-ray photography. Simak (42) reported that reduction in seed quality by insect damage could be measured exactly by x-ray photography methods. In later years Gustafson *et al.*

(17) disclosed the fact that by means of x-ray photography alone it was not possible to distinguish between germinable and non-germinable seeds. A seed material with well developed embryo and endosperm and normal x-ray absorption could be dead owing to an unstable storage, heat treatment of some kind or ageing.These conditions could not be detected on the x-ray photographic plates. The authors tried to improve the technique by using contrast agents i.e. by pre-impregnating the seeds with salts of heavy cations. It was observed that the status of the seed could be detected on the x-ray film by this improved method. The cations barium, silver, lead, etc. absorb x-radiation strongly. In perfectly sound seeds the salts could not penetrate into the interior and, therefore, the embryo and endosperm would look identical whether the seeds were impregnated or not. The picture is different in dead and semi-viable seeds in which the places of penetration of the contrast agent is visible on x-ray photographic plates. Judging from the amount of penetration and impregnation of individual seeds the viability of the specific seed material could be concluded. Simak (43) working with Pinus silvestris, reported similar results. Kamara (24) reported that for Pinus, barium chloride and for Picea, organic substances like urografin and umbradil were suitable contrast agents.

From the experiments he concluded that the percentage of mechanically damaged seeds in Pinus could be determined through the use of organic chemicals as contrast agents.

Some of the early attempts to develop rapid viability tests based on individual seed responses were concerned with materials which would color dead seeds or portions of dead seeds.

Dimitriewicz (10) published the results of his attempts to find such a method. He treated halved grains of Hordeum vulgare with sulphuric acid and obtained different color reactions between vital and weakened germs. Neljubow (34) found that indigo carmine penetrated dead tissue but did not readily penetrate living tissue. Embryos were cut through or excised and soaked in a weak solution of indigo carmine for one hour then washed and evaluated. Interpretation was based principally upon the proportion of the embryo remaining uncolored (uncolored or slightly colored embryos were considered best for germination).

Plaut and Halfon (37) used resazurin, an indicator used in milk testing, for staining seeds. They observed that Pisum sativum, Vicia sp. and Cucumis sativus seeds respond to treatment with resazurin solution. The absorption of the substance by the seed was physical and only the reaction of the radicle could be used as a

reliable indicator. The radicles of the dead and living seeds do not react equally to the solution. In dead seed, the blue color of the resazurin was absorbed and remained unchanged, whereas, in the living seeds the resazurin undergoes changes. As a result of the enzymatic activities of the seed, and is converted to resorufin, which is pink and this in turn changes into dihydroresorufin which is colorless. This latter process is reversible. Resazurin has an advantage as it is one of the staining substances which does not harm the seeds and the seeds are capable of germination after immersion in it. This enables the results obtained by the staining method to be checked by subsequent germination of the treated seed in sand. Plaut et al. (38) reported that estimation of germinability by resazurin was almost correct compared to a control or germination after resazurin test.

Several workers tried to develop methods based upon enzymatic activities. According to Delouche et al. (8) several workers like McHarque, Davis, Laggatt, Turesson and others worked on this method. McHarque developed a physiological test for the enzyme peroxidase. Davis and Laggatt measured the activity of the enzyme catalase as an indicator of viability of seed.

According to Delouche (8) Turesson was the first

to work with a group of enzymes, the dehydrogenases, which are involved in oxidation-reduction reactions of many organic compounds. The reduced and oxidized forms of some of these compounds were characteristic by different colors. Consequently it was an easily observed phenomenon involving a dramatic color change. Hasegawa (18) based the viability test on the reduction of telluric and selenic salts. He reported that a viable embryo will reduce the colorless tellurium and selenium salts into their basic colored forms. A uniform darkish indigo and black color will appear on, and inside, the embryo of viable seeds.

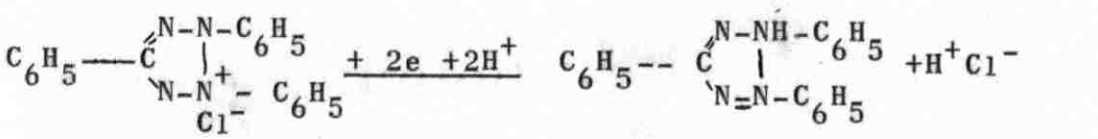
Gadd and Kjaer (13) believed that the selenite method was no good as it implied the inaccuracy that tissue remain unstained because of the inability of the selenium salts to penetrate deep into the tissue. They suggested a double drying method by staining presoaked longitudinally cut seeds in a mixture of equal parts of one percent solution of sodium hydrogen selenite and 0.25 percent of indigo carmine for twenty-four hours. They reported that by this method the seeds would be stained simultaneously with two different colors - the live part would turn red and the dead part blue, thus facilitating evaluation.

Gadd (12) developed a viability test for peas

based on the decoloration of malachite green by dehydrogenases.

During world war II, Lacon (26) developed the tetrazolium method for seed testing. Many tetrazolium salts such as 2, 3, 5-triphenyl tetrazolium chloride (TTC), Tetrazolium red (TR), 2-(P-iodophenyl)-3-(P-nitrophenyl)-5- phenyl tetrazolium chloride (INT), Tetrazolium violet (TV), Neo-tetrazolium (NT) and Tetrazolium blue (TB) are available and can be used for testing seed. The one which is most commonly used, is 2, 3, 5-triphenyl tetrazolium chloride. It is a white powder and colorless in solution (15).

The tetrazolium salt is an oxidation-reduction indicator and forms carmine-red formazan upon reduction. The development of a non-diffusable red color in a specific tissue is the result of reduction of the chemical by enzymatic action and is an indicator of the presence of an active respiratory process. One or more of the dehydrogenase systems appear to be involved in the reaction (8, 39). The reaction looks like this:



2,3,5-triphenyl tetrazolium chloride colorless

Formazan red

The dehydrogenase enzyme systems are involved in the respiratory activity of biological systems. Hydrogen ions are transferred to tetrazolium which acts as a hydrogen acceptor and thus the reduction of the salt takes place. There is a sharp color difference between viable and nonviable tissue. The former takes a non-difusable red color due to the formation of formazan while the latter retains its natural color (8).

A wide range of concentrations may be used with equal success. The most commonly used concentrations are 1, 0.5 and 0.1 percent. In general, the higher concentrations are used for legumes, cotton and small seeded grasses which are not bisected (8, 15). The solution is made by dissolving the salt in water. It can be stored in the dark or in amber colored bottles at room temperature for many weeks (15).

The new methods of the tetrazolium test are a little bit different from those recommended by Lacon (26). According to the latter the embryo in most cereals must be removed from the kernel before staining, with the exception of maize and oats. In maize a longitudinal section was all right but in other cases it was not considered to be sufficient as the lateral root primordia could not be seen. In oats, due to the thin pericarp, excision was not necessary. His recommendation for the

concentration of the solution to be used was one percent for excised embryos or other seed sections. For time, the recommendation was seven to eight hours at room temperature. The speed of the reaction is increased by many factors e.g. presoaking, higher temperature, vacuum, high pH and higher concentration of the solution (15).

Lacon (26) is of the opinion that the tetrazolium test practically eliminates experimental error. Statistical analyses of several thousand tests have shown that in tetrazolium tests with 200 kernels each, the errors always were lower than those in actual germination tests with 400 kernels. He pointed out the following advantages of this test:

1. No great amount of space and complex apparatus is required.
2. Execution is rapid even for large scale tests.
3. The method provides reliable and exact results.
4. "Germination potency" (Germinable+dormant) can be determined.

Delouche et al. (8) pointed out that inspite of the advantages, this test has some serious limitations too:

1. Although this test requires a relatively short period of time, the test generally requires more total man hours of work than does the germination test.

2. Some of the test techniques are extremely tedious and require both patience and experience.
3. Tetrazolium tests do not give proper information, about hard seed and/or dormant seed.
4. Since the tetrazolium test does not involve germination, microorganisms harmful to germinating seedlings are not detected.

Porter (39) made a comparison between tetrazolium and germination tests using Zea mays, Hordeum vulgare, Avena sp., Secale cereale, Triticum sp., Pisum sativum, Glycine max,, Vicia sp., Gossypium sp., and Polygonum convolvulus. In most of the cases there was agreement in the results. In only a few cases there were differences but these were not great.

Shuel (41) tested the method with Hordeum vulgare, Avena sativa and Triticum sp. and reported that this test gave a reliable index of germinability with new seed, but with old seed,whose viability was likely to be less than about sixty percent, this test was inaccurate.

Bennett and Loomis (2) reported that freezing injury to seed corn could be estimated with fair accuracy provided the germination was high and the corn had been stored for some time after freezing. By the tetrazolium test it was not possible for them to estimate the percentage of abnormal seedlings. Freshly frozen immature

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corn of thirty to sixty percent moisture gave more intense staining and they ascribed this increased intensity of staining to increased permeability of the injured cells to the dye which was reduced to the colored form by substances present in the tissue before freezing. They concluded, however, that by experience it was possible to estimate the injured seeds immediately after freezing. Goodsell (14) in a similar experiment with frozen seed corn reported that high positive correlation was found between tetrazolium readings and germination percentages. In most of the cases the tetrazolium test tended to give a high estimate of germinability (2, 14, 41). Goodsell suggested that the germination percentage should be regarded as 95 percent of the tetrazolium readings.

Iseley (21) who worked with Avena sp., Triticum sp., Hordeum sp., and Secale sp., concluded that reasonably accurate assays of viability could be made within a few hours with the tetrazolium test. The staining technique might in some cases over-estimate the value of the seed. Several workers (4, 29) reported that significant correlations were obtained between results of the tetrazolium ratings and both germination and rate of emergence tests in cotton seed. Therefore, it was felt that tetrazolium ratings could provide some measurement of relative vigor of cotton seed. Stain characteristics and

conditions of seed tissue should be the main basis of ratings.

Injurious influences of artificial seed drying have been reported on recently at different times. Bulat (3) reported that it was possible, with the tetrazolium test to detect clearly the treatment injuries in artificially dried seeds. The types of injuries and their extensiveness could be noticed in the tetrazolium technique in the form of layer like necrosis of the radicle. The non-treated control appeared free of the necrosis. Hot water treatment for seed disinfection parallels the problem of artificial drying of seed, which provide material of specific embryo injuries in as much as such arose from it. The hot water injuries could be detected in the tetrazolium test with greater accuracy.

Cinki (5) measured the relationship of germination capacity (under laboratory and green house conditions) and viability, estimated by tetrazolium tests, with different treatments and storage conditions. High correlation was observed between germinability and the tetrazolium test. Low correlation was found for seed samples stored under desert conditions ($90^{o}F$ with 40 percent relative humidity) for different lengths of time . These differences were attributed to the overdrying effect of the environment. A modification of the tetrazolium

test procedure was suggested in order to get better results. A modification such as slow hydration of the seed under saturated moisture conditions could probably improve the results.

Cobb (6) reported that the use of the tetrazolium test as a viability indicator for seeds that have been injured or killed by fumigating with methyl bromide did not show the usual correlation of the tetrazolium staining with laboratory germination. The tetrazolium staining test percentages corresponded closely to the laboratory germination values that existed prior to methyl bromide fumigation. The use of this biochemical test was, therefore, suggested as a means of estimating the germination percentage that existed prior to injury by methyl bromide fumigation.

The fact that vigor is an important quality characteristic in seeds, has been given great attention by many investigators. Iseley (22) pointed out that two views predominate in most concepts of vigor: (a) susceptibility to unfavourable conditions, (b) vigor *per se* as reflected in speed of germination and rapidity of growth rate of seedlings. He further stated that these may be regarded as separate entities or as facets of a single physiological complex. He also categorized the vigor tests into two types: (a) direct tests which

simulate pertinent unfavourable field conditions on a laboratory scale and (b) indirect tests which measure certain physiological attributes of seed. The use of the tetrazolium test as a means of evaluating vigor is one of the indirect types which have received considerable attention within recent years.

Rogler (40) reported that seed size and weight have distinct relationships with the seedling vigor in Agropyron cristatum. Highly significant differences were found between total seedling emergence, as affected by depths, weights and the interaction of depths and weights was also significant. Kneebone (25) reported similar results with some native grasses.

Tempe (46) used the hot-water treatment for evaluating the degree of weakness of different crop seed samples, but finally the method was not recommended for routine testing. According to Moore (30, 31, 32) possible seed testing approaches to "goodness" or seed quality are largely resolved into two distinctly different areas (a) vigor test (b) quick tests. The tetrazolium test provides a more critical method for measuring seed quality on the basis of soundness. This test completely bypasses the adverse influence of environment on germination and seedling development. In addition, he mentioned that the tetrazolium test permits a detailed

evaluation of individual structures within individual seeds and that classification of seeds for vigor was made possible by examining variations in staining patterns and by studying the locations and nature of non-stained areas. Cell turgidity and other characteristics should be taken into consideration. Careful observation of tetrazolium staining patterns reveal seed weakness is not detectable in the standard germination test (4, 30, 31, 32) and that natural ageing (33) and mechanical injury (5, 33) are detectable.

Delouche et al. (9) mentioned that Rice studied the evaluation of vigor in corn with the tetrazolium test as compared with other methods like the cold test, the standard germination test, etc. Employing the tetrazolium test he found that stain intensity obtained in corn in a specific time providedas precise a measure of vigor as the cold test.

Bulat (4) evaluated the vigor of seventeen differently treated samples of cotton seed in parallel experiments with the germination and tetrazolium tests. The stained seeds were classified into ten groups according to the spread of the necrotic areas. The embryos of groups one to four showing little necrosis were considered viable. It was concluded that the reduced seed vigor, primarily due to the occurrence of necrosis could

be determined in the tetrazolium test.

Although the tetrazolium testing technique is simple, it needs considerable knowledge about the structure of the individual seeds. Correct interpretation depends on the knowledge of functions and structures within the seed. The beginner in using the tetrazolium test will frequently under or over-estimate the germination percentage. This can only be overcome by practice. With practice confidence increases. An analyst cannot begin to apply this test routinly until he has confidence in himself and in the test (8).

MATERIALS AND METHODS

The present investigation was conducted in the Seed Technology Laboratory of the American University of Beirut during the years 1964 and 1965. Six different crop seeds were used in this study. These were the following: three varieties of corn (Zea mays), one variety of sorghum (Sorghum vulgare), one variety of barley (Hordeum vulgare), three varieties of wheat, two of Triticum aestivum and one of Triticum durum, one variety of common vetch (Vicia sativa) and two varieties of cotton (Gossypium sp.). New and old seed samples of these crops were obtained from the Agricultural Research and Education Center except for the cotton seed which was obtained from Pakistan.

Germination and Tetrazolium Test

In order to have seeds of different germinating capacities a fraction of each seed sample was exposed to unfavorable environmental conditions of high moisture and temperature to decrease its viability. The viability was decreased to different levels in the laboratory by means of adding different amounts of water to the seed and then exposing it to 40°C for different periods of

time,usually between seven and fourteen days. Directly after this treatment, the seeds were mixed thoroughly before being divided into sub-samples for laboratory germination and tetrazolium tests.

Duplicate samples of 100 seeds were used for each of the laboratory germination and tetrazolium tests. The germination tests were carried out according to the specifications of the International Seed Testing Association (1). The tetrazolium tests,however,were conducted according to the following procedure:

1. The seeds of wheat, barley and vetch were soaked in water for three to four hours. The seeds of corn, sorghum and cotton were soaked for five to six hours.
2. The graminaceous seeds were bisected longitudinally and medially through the middle of the embryo. In each case one half of the seed was taken for staining and the other half was discarded.
3. The whole seed of vetch was used for staining.
4. After soaking the cotton seeds for five to six hours the seed coats were removed and then the seeds were resoaked for another additional hour to remove the papery membrane around the embryo before staining.

The concentrations of the tetrazolium staining solutions used were 0.1 percent for the cut graminaceous

seeds and 1.0 percent for the vetch and cotton seed. Enough amount of the tetrazolium solution for staining was poured over the seeds in petri dishes. The time that was used for staining the different crop seeds varied between 2 and 2½ hours at a temperature of 40°C. After the staining period was over, the excess tetrazolium chloride solution was drained off and the material was washed in distilled water several times. Enough water was left in the petri dishes after the final washing to prevent drying of the embryo. After the staining procedure every individual seed was examined under a magnifying glass or the low power of a binocular microscope. The staining results were recorded and later tabulated.

Vigor Test

Seeds were germinated in sterilized sand in aluminium pans and the length of the seedlings above the sand level was taken as a measure of vigor on the assumption that vigorous seedlings grow taller than do the less vigorous ones. In addition, the cold test was used as a measure of vigor for corn and sorghum. In this test of vigor the seeds were planted in nonsterilized soil obtained directly from the field and then exposed to a low temperature of 2 to 3°C for a period of seven days

before the seeds were transplanted to the optimum temperature for germination.

With respect to the tetrazolium test, seed vigor was studied on the basis of the intensity of staining as well as the extent of necrosis on the embryonic tissue.

Detection of Chemical Injury

In another experiment chemical injury was inflicted on the seeds using mercuric chloride, a chemical commonly used for disinfection of seed and Panogen-15, an organic mercuric seed treatment compound. Mercuric chloride was used on barley seeds only but Panogen-15 was used on wheat, barley, corn, sorghum, cotton and vetch. The concentrations that were used to cause injury to the seeds were 1.0 percent of Panogen-15 and 50 percent of the mercuric chloride stock solution (1000 c.c. of stock solution contained 20 g of mercuric chloride and 28.6 c.c. of concentrated hydrochloric acid and the rest was water). The different periods used for soaking the seed in mercuric chloride and Panogen-15 were the following:

1. Three and four minutes soaking of barley seed in mercuric chloride solution.
2. Five, thirty and sixty minutes of soaking of wheat, barley, and sorghum in Panogen-15 solution.
3. Ten, thirty and sixty minutes soaking of corn in

Panogen-15 solution.

4. One, two and five hours soaking of vetch in Panogen-15 solution.
5. Two and five hours soaking of cotton in Panogen-15 solution.

Chemical toxicity of the seed was studied by (a) germinating the seeds in the laboratory and recording the toxicity symptoms of the seedlings and (b) the tetrazolium test. With respect to the latter, chemical injury was identified on the basis of staining pattern, development of abnormal coloration and other peculiarities in the stained embryos.

Interpretation of Tetrazolium Staining Results

These seeds which had at least the epicotyle, the zone of the seminal roots, and the greater part of the scutellum stained were considered viable in the germinaceous seeds (Figures 1, 2 and 3). Staining of the radicles was not considered important as plants belonging to the grass family do not depend on primary roots as they do on seminal roots. After staining, the seed coats of the vetch seeds were removed to expose the embryo in order to help in the interpretation of the results. Those seeds were considered viable, in which the radicle, the plumule, and at least one half of each of the cotyledons

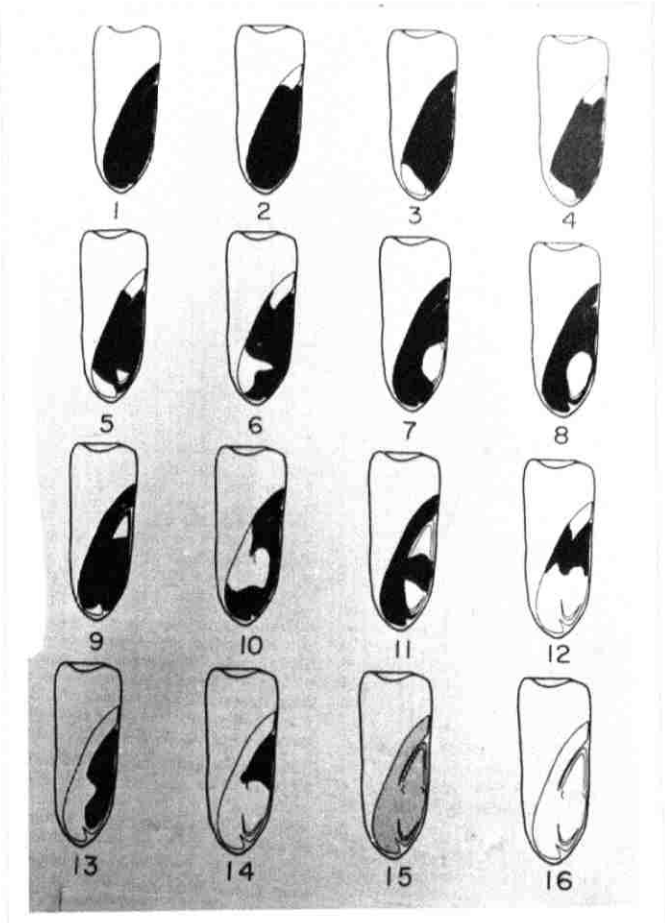

Figure 1. Criteria for interpreting the tetrazolium test results on corn seed. Black area indicates stained and living tissue, white areas represent unstained and dead tissue, and the shaded area lightly stained tissue. (8).

Nos. 1 - 6. Germinable.
Nos. 7 -16 Nongerminable.

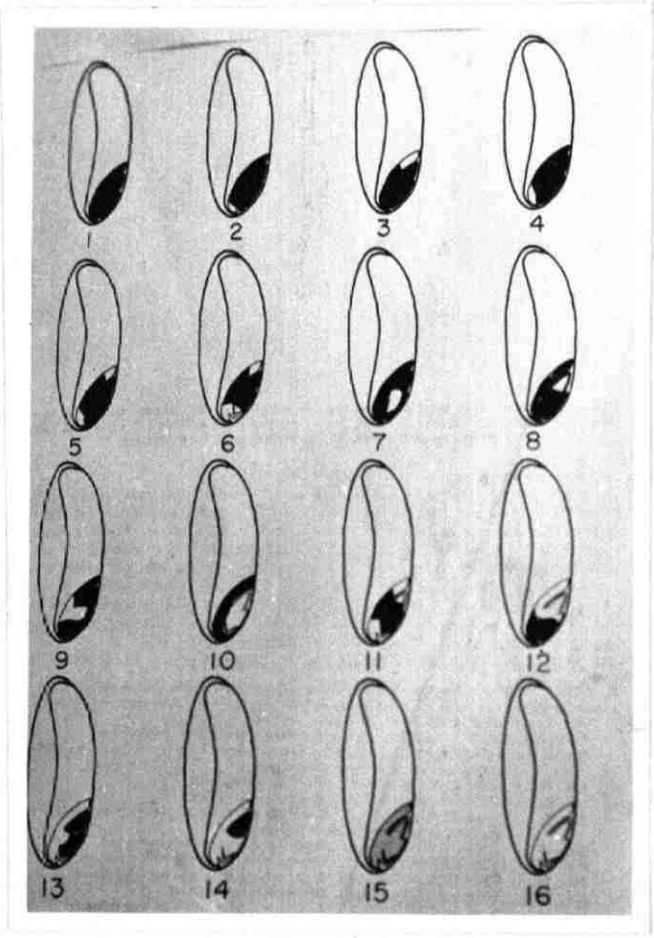

Figure 2. Criteria for interpreting the tetrazolium test results on wheat and barley seeds. Black areas indicate stained living tissue, white areas represent unstained and dead tissue, and the shaded areas represent lightly stained tissue. (8).

Nos. 1 - 6 Germinable.
Nos. 7 -16 Nongerminable.

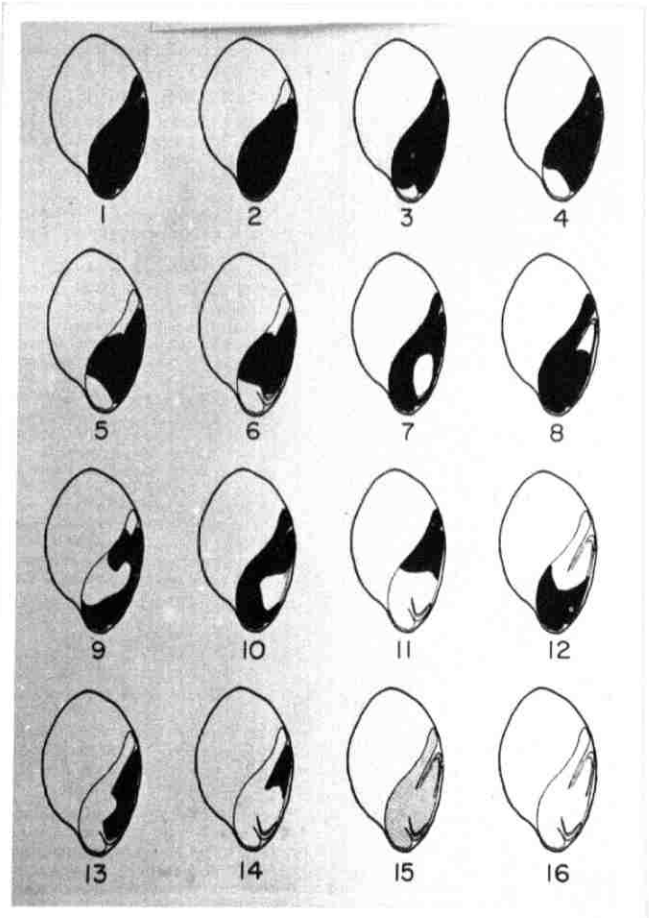

Figure 3. Criteria for interpreting the tetrazolium test results on sorghum seed. Black areas indicate stained living tissue, white areas represent unstained dead tissue, and the shaded areas represent lightly stained tissue. (8).

Nos. 1 - 6 Germinable.
Nos. 7 -16 Nongerminable.

were stained (Figure 4). Seeds in which the extreme tip of the radicle was unstained were also considered viable. The criteria of viability in cotton (Figure 5) are almost the same as in vetches except that seeds in which more than one third of the cotyledonary tissue was necrotic were considered nonviable.

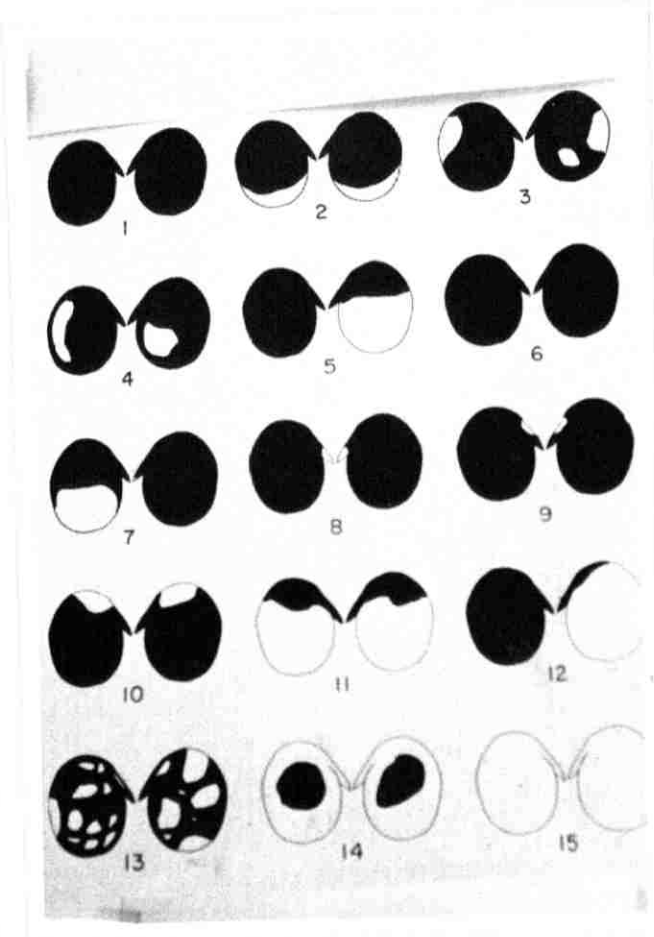

Figure 4. Criteria for interpreting the tetrazolium test results of vetch seed. Illustrations are paired and depict both sides of seed. Black areas indicate stained living tissue, white areas represent unstained and dead tissue. (8).

Nos. 1 - 7 Germinable.
Nos. 8 -15 Nongerminable.

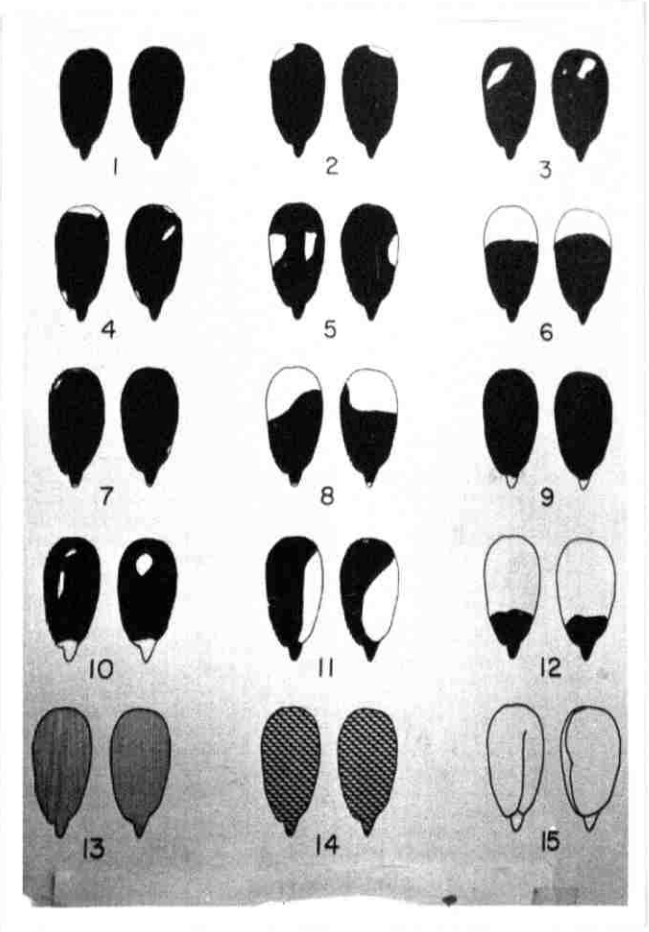

Figure 5. Criteria for interpreting the tetrazolium test results on cotton seed. Illustrations are paired and depict both sides of the seeds. Black areas indicate stained living tissue, white areas represent unstained and dead tissue, lightly shaded areas represent lightly stained tissue, and deeply shaded areas represent stained with abnormal color. (8).

Nos. 1 - 7 Germinable.
Nos. 8 -15 Nongerminable.

RESULTS AND DISCUSSION

Tetrazolium Test vs. Germination Test

The germination and tetrazolium tests have been compared in the first part of this study. The "germination tolerances" which are recognized by the International Seed Testing Association (1) as latitudes of variation for comparing the germination results between two experiments were employed. The results of the two tests are shown in Tables 1 to 6 on the samples of corn (Zea mays), wheat (Triticum spp.), barley (Hordeum vulgare), sorghum (Sorghum vulgare), cotton (Gossypium sp.) and vetch (Vicia sativa). The tolerances reported in the tables are taken from the germination tolerances in the appendix (Table 17) for the convenience of comparison. In the germination test the percentages of normal seedlings were compared with the percentages of viable seed obtained by the tetrazolium test. The results reported in all the tables are averages of two replications of 100 seeds each. The results of the germination and tetrazolium tests of corn (Table 1), wheat (Table 2), sorghum (Table 4) and vetch (Table 6) fall within the tolerance range. The percentages of both tests in barley

(Table 3) and cotton (Table 5) were also shown to be comparable except for the barley sample number two at the fourth and sixth levels of deterioration and cotton sample number two at the fourth level of deterioration where the results exceeded the tolerance range. The tetrazolium test as appeared from the results may be used successfully for the determination of viability of the crop seeds used in this study. These results are in agreement with a group of investigators (5, 14 and 39) who believe that the tetrazolium test may be used successfully to evaluate the germination of different crop seeds. Bennett (2), Goodsell (14) and Shuel (41) reported that in most cases the tetrazolium test tended to give a high estimate of germinability. Goodsell (14) suggested that this germination percentage should be regarded as 95 percent of the tetrazolium readings. It is clear, however, from the results reported in this study that the use of tetrazolium chloride did not show any tendency for over-estimating seed viability. The results also did not agree with Shuel (41) who believed that seeds whose viability was less than sixty percent would give very inaccurate results with the tetrazolium test.

Table 1. Comparative results of germination and tetrazolium tests on different samples of corn seed.

Sample number	Year of harvest	Tetrazolium test		Germination test			Germination tolerance+
		% viable	% nonviable	% normal	% abnormal	% dead	
1	1964	100	0	100	0	0	4
2	1962	94	6	98	0	2	4
3	1959	96	4	97	0	3	4

+ The given germination tolerances are recognized by the International Seed Testing Association as latitudes of variation for comparing two germination tests (Table 17).

Table 2. Comparative results of germination and tetrazolium tests, at different levels of deterioration, of three varieties of wheat.

Varieties	Levels of deterioration[+]	Tetrazolium test		Germination test			Germination tolerance
		% viable	% nonviable	% normal	% abnormal	% dead	
Florence aurore	1	90	10	90	5	5	6
	2	81	19	82	4	14	7
	3	67	33	65	4	31	9
	4	54	46	57	5	38	10
Mishri-quani	1	75	25	80	10	10	7
	2	68	32	64	14	22	9
Hourani	1	91	9	94	5	1	6
	2	79	21	83	4	13	7
	3	53	47	46	7	47	10

[+] The levels of deterioration of seed samples were obtained by means of adding different amounts of water to the seed and then exposing it to 40°C for seven days. The numbers one, two, etc. are in the increasing order of the severity of conditions of deterioration.

Table 3. Comparative results of germination and tetrazolium tests at different levels of deterioration of three samples of barley.

Sample number	Levels of deterioration[+]	Tetrazolium test		Germination test			Germination tolerance
		% viable	% nonviable	% normal	% abnormal	% dead	
1	1	94	6	97	1	2	4
	2	83	17	86	2	12	7
	3	75	25	74	2	24	8
	4	15	85	10	-	90	10
	5	5	95	5	-	95	10
2	1	93	7	97	2	1	4
	2	88	12	93	5	2	6
	3	85	15	90[++]	4	6	6
	4	81	19	89[++]	7	4	7
	5	81	19	73[++]	6	21	8
	6	69	31	58[++]	6	36	10
	7	44	56	51	4	45	10
	8	26	74	16	3	81	10
3	1	84	16	90	8	2	6
	2	51	49	59	10	31	10
	3	44	56	41	4	55	10

[+] The levels of deterioration of seed samples were obtained by means of adding different amounts of water to the seed and then exposing it to 40°C for seven days. The numbers one, two, etc. are in the increasing order of the severity of conditions of deterioration.

[++] Exceeded the tolerance range.

Table 4. Comparative results of germination and tetrazolium tests at different levels of deterioration in sorghum seed.

Levels of deterioration[+]	Tetrazolium test		Germination test			Germination tolerance
	% viable	% nonviable	% normal	% abnormal	% dead	
1	75	25	79	18	3	8
2	74	26	76	17	7	8
3	72	28	75	9	16	8
4	69	31	73	11	16	8

[+] The levels of deterioration of seed samples were obtained by means of adding different amounts of water to the seed and then exposing it to 40°C for seven days. The numbers one, two, etc. are in the increasing order of the severity of conditions of deterioration.

Table 5. Comparative results of germination and tetrazolium tests at different levels of deterioration in two selections of cotton seed.

Selection and sample number	Levels of deterioration[+]	Tetrazolium test		Germination test			Germination tolerance
		% viable	% nonviable	% normal	% abnormal	% dead	
L.S.S. Sample 1	1	85	15	86	1	13	7
	2	83	17	77	-	23	8
	3	82	18	79	-	21	8
	4	79	21	81	1	18	7
	5	77	23	77	5	18	8
	6	67	33	63	4	33	9
L.S.S. Sample 2	1	90	10	86	12	2	7
	2	83	17	85	12	3	7
	3	82	18	76[++]	11	13	8
	4	81	19	71	8	21	8
	5	78	22	70	10	20	8
	6	76	24	68	7	25	9
	7	68	32	62	7	31	9
M4	1	39	61	38	1	61	10
	2	32	68	37	13	50	10
	3	30	70	35	9	56	10
	4	36	62	34	11	55	10
	5	32	68	30	12	58	10
	6	32	68	30	13	57	10
	7	25	75	22	9	69	10
	8	28	72	21	18	61	10

[+] The levels of deterioration of seed samples were obtained by means of adding different amounts of water to the seed and then exposing it to 40°C for seven days. The numbers one, two, etc. are in the increasing order of the severity of conditions of deterioration.

[++] Exceeded the tolerance range.

Table 6. Comparative results of germination and tetrazolium tests of two samples of vetch seed.

Sample number	Levels of deterioration[+]	Tetrazolium test		Germination test			Germination tolerance
		% viable	% nonviable	% normal	% abnormal	% dead	
1	1	96	4	92	1	7	6
	2	91	9	91	-	9	6
	3	81	19	84	1	15	7
	4	85	15	83	2	15	7
	5	68	32	74	4	22	8
	6	42	58	48	7	45	10
2	1	95	5	96	0	4	5
	2	94	6	95	2	3	5
	3	95	5	91	2	7	6
	4	86	14	87	8	5	7
	5	83	17	81	13	6	7
	6	30	70	27	57	16	10
	7	6	94	8	60	32	10

[+] The levels of deterioration of seed samples were obtained by means of adding different amounts of water to the seed and then exposing it to 40°C for seven days. The numbers one, two, etc. are in the increasing order of the severity of conditions of deterioration.

Tetrazolium Test and Seed Vigor

The evaluation of seed vigor has been overlooked in the present official laboratory germination tests although it is one of the most important factors of seed quality. Seed vigor is not only a measure of the capacity of seed to survive and emerge under adverse field conditions but is also a measure of the storability of the seed. Seeds low in vigor are as susceptible to adverse storage conditions as they are to adverse field conditions. In the present study, seed vigor was measured by the tetrazolium test on the basis of intensity of coloration and staining patterns of the embryo. The tetrazolium results were compared with the vigor results obtained by the cold test on corn and sorghum and by the length of the seedlings at the final germination count on barley, cotton and vetch.

The cold test was used for detecting vigor in three samples of corn. As shown in Table 7, the three samples whose seeds were harvested in 1959, 1962 and 1964 gave similarly high germination results of 97, 98 and 100 percent, respectively. Although the seeds did not show much variation in germinability, marked differences, however, were observed in the cold test results. The germination percentages of the seed samples harvested

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Table 7. Comparison of germination test, cold test and tetrazolium test results in three samples of corn.

Sample number	Year of seed harvest	Germination test			Cold test	Tetrazolium test			
		% normal	% abnormal	% dead	% germination	% viable			% nonviable
						deep stained	light stained	total	
1	1964	100	0	0	88	90	10	100	0
2	1962	98	0	2	73	64	30	94	6
3	1959	97	0	3	32	50	46	96	4

in 1959, 1962 and 1964 were brought down to 32, 73, and 88, respectively. Only the vigorous seeds were able to germinate and survive the unfavourable conditions of the cold test.

The tetrazolium test was employed to test seed vigor by referring to the deeply and uniformly stained seeds as vigorous and the lightly stained as less vigorous. In sample one of the 1964 crop seeds, the percentage of deeply stained seeds is in fair agreement with that of the cold test results. In sample two, the cold test result was higher than was the percentage of deep stained seeds in the tetrazolium test. This condition is just the opposite in sample three where the cold test gave 32 percent germination compared to 50 percent of deep stained in the tetrazolium test. It should be mentioned here that the seeds of sample two were previously treated with some chemical seed protectant which may have protected the seeds from microbial attack in the cold test and resulted in a higher germination percentage. The higher percentage of deeply stained seed in the tetrazolium test in the third sample compared to the cold test results may be due to the limitation of the tetrazolium test to give accurate results with low viability. Shuel (41) mentioned that the tetrazolium test fails to give accurate results with seeds whose germinability is less

than 60 percent, but did not specify the limitation of tetrazolium chloride when used for testing vigor of seeds low in viability. According to the results of this study Shuel's statement seems to hold true when the tetrazolium test is used for vigor rather than viability.

Although the cold test is a specific test for corn, it was also used in this study to evaluate vigor in sorghum samples on the assumption that less vigorous seedlings will not be able to stand unfavorable conditions of this test. It can be seen from the results shown in Table 8 that the germinability of the samples was not much lowered by the different levels of deterioration although vigor was. The decrease in germination under the cold test conditions was inversely related to the increase in the deterioration level. The deep and perfectly stained viable seeds in the tetrazolium results do not compare closely with the cold test results. The fact that the results of the cold test at the first two levels of deterioration were very close to the germination results indicates that the cold test is either inapplicable to sorghum for measuring vigor or that most of the germinable seeds were vigorous. In the case of the tetrazolium test for vigor, the case was different in that 13 percent and 16 percent of the germinable seeds of

Table 8. Comparison of germination test, cold test and tetrazolium test results at different levels of deterioration in sorghum seed.

Levels of deterioration[+]	Germination test			Cold test	Tetrazolium test			
	% normal	% abnormal	% dead	% germinable	% Viable: deep stained	% Viable: light stained	% Viable: total	% nonviable
1	79	18	3	74	62	13	75	25
2	76	17	7	69	58	16	74	26
3	75	10	15	65	59	13	72	28
4	73	11	16	58	56	13	69	31

[+] Seeds were deteriorated to different levels by means of adding different amounts of water and then putting the moist seed samples at 40°C for seven days: The numbers from one to four are in the increasing order of the severity of conditions of deterioration.

samples one and two, respectively (Table 8) were grouped in the less vigorous (light stained) category. The percentages of the vigorous (deep stained) seed were considerably lower than the percent germinable under the cold test conditions. It can be suspected from these results that the cold test, which is not really a test of vigor for sorghum, has overestimated vigor and that the tetrazolium results were more conservative.

The seed vigor results as measured by the seedling length are shown in Tables 9, 10, and 11. The normal seedlings were divided by an arbitrary scale into two groups: vigorous and less vigorous. Seedlings of less than five centimeters in length were considered less vigorous and those of five centimeters and above were considered vigorous. As in corn and sorghum the viable seeds of barley in the tetrazolium test were also devided into two groups: the deep stained or vigorous and the light stained or less vigorous. The results of the barley vigor test are shown in Table 9. It seems from the results that the deep and light stained seeds are in good agreement with the two arbitrary vigorous and less vigorous seedling groups, respectively. It can be noticed that the total number of viable seeds according to the tetrazolium test of each level of deterioration also corresponds with the total number of normal seedlings in

Table 9. Tetrazolium and laboratory germination test results for a comparative study of vigor in barley seed.

Levels of deterioration+	Tetrazolium test % viable seed			Germination test % normal seedling		
	deep stained	light stained	total	below 5 cms.	above 5 cms.	total
1	82	12	94	9	88	97
2	60	23	83	23	63	86
3	5	9	14	7	2	9
4	1	4	5	5	0	5

+ Seeds were deteriorated to different levels by means of adding different amounts of water and then putting the moist seed samples at 40°C for seven days. The numbers from one to four are in the increasing order of the severity of conditions of deterioration.

the germination test.

In cotton there was no visible difference of the staining intensity in the viable seeds in the tetrazolium test, but there were prominent necrotic areas on the radicle and on the cotyledonary tissue. It can be seen from the results in Table 10 that the number of seeds having necrosis on the embryonic tissue, varied directly with seed deterioration. Probably these necrotic spots on the embryonic tissue, specially at the root tip, are among the real causes of retarded growth of seedlings. The tetrazolium test appeared to be helpful here in detecting these necrotic areas. Bulat (4) reported similar results on seventeen cotton seed samples of different viability levels.

In vetch, although differences in vigor were distinctly observed in the germination test of the different samples, it seemed very difficult to detect it in the tetrazolium test. Practically there was no difference in the tetrazolium test results (Table 11) among the viable seeds of the six samples used in the experiment. Neither the staining intensity nor the staining patterns could be used for detecting vigor in this seed. These results are not in agreement with Moore (32), who believes that this test can be used in detecting vigor in corn, vetch, and many other crop seeds.

Table 10. Tetrazolium and laboratory germination test results for a comparative study of vigor in cotton seed.

Selection	Levels of deterioration+	Tetrazolium test % viable seed				Germination test % normal seedling		
		perfectly stained	necrosis on cotyledon	necrosis on radicle	total	below 5 cms.	above 5 cms.	total
L.S.S.	1	83	2	0	85	1	85	86
	2	74	5	3	82	16	63	79
	3	65	8	6	79	19	62	81
	4	56	4	17	77	27	50	77
	5	33	1	33	67	28	35	63
M_4	1	32	7	0	39	5	33	38

+ Seeds were deteriorated to different levels by means of adding different amounts of water and then putting the moist seed samples at 40°C for seven days. The numbers from one to five are in the increasing order of the severity of conditions of deterioration.

Table 11. Tetrazolium and laboratory germination test results for a comparative study of vigor in vetch seed.

Levels of deterioration[+]	Tetrazolium test % viable seed			Germination test % normal seedling		
	perfectly stained	not perfectly stained	total	below 5 cms.	above 5 cms.	total
1	94	2	96	1	91	92
2	88	3	91	2	89	91
3	81	0	81	12	73	85
4	80	2	82	11	72	83
5	64	3	65	9	65	74
6	41	1	42	11	37	48

[+] Seeds were deteriorated to different levels by means of adding different amounts of water and then putting the moist seed samples at 40°C for seven days. The numbers from one to six are in the increasing order of the severity of conditions of deterioration.

Tetrazolium Test and Chemical Toxicity

The benefits of seed treatments and seed disinfectants by different chemicals against seed borne microorganisms have been well established for a long time. Sometimes there chemicals result in different levels of toxicity for the seed itself. So an experiment was designed to find out if tetrazolium chloride can be used to detect such toxicity in the seed. For this purpose, mercuric chloride (HgCl) was used on barley seeds and Panogen-15 on the seeds of wheat, barley, corn, sorghum, cotton and vetch.

Mercuric chloride injury in barley seeds

The study of germination results in Table 12 indicates that the two treatments of mercuric chloride have caused injury in the barley seed. More abnormal and dead seeds were observed in the mercuric chloride treated samples as compared to the non-treated ones. The percent abnormal and percent dead varied directly with the time the seeds were soaked in the mercuric chloride solution. Of all the seedlings that were considered normal most of them were weak and yellow compared to the vigorous and green of the non-treated ones. The tetrazolium test that was used along with the germination test failed to detect the toxicity symptoms caused by the chemical and observed by the germination test. Since the injury was not detected

Table 12. Tetrazolium and germination test results of barley seeds with induced toxicity by mercuric chloride.

Treatment (40°C)		Tetrazolium test		Germination test		
Chemical	Time of soaking	% viable	% nonviable	% normal	% abnormal	% dead
Distilled water	three minutes	89	11	94	3	3
HgCl	three minutes	86	14	85	8	7
HgCl	four minutes	86	14	76	11	13

by the tetrazolium chloride, this is therefore, an indication that such chemical injury has occurred on certain sites of germination other than the dehydrogenases. This observation shows the limitation of the tetrazolium chloride test as a means of testing the different sites and factors that influence seed germination.

A one percent solution of Panogen-15 was found to cause injury in different seeds treated for different periods of time.

Panogen-15 injury in corn seed

Toxicity due to this chemical was identified in the germination test (Table 13) . Low germination percentages and high abnormal and dead seeds are shown in treated samples. The percentages of the abnormal corn seedlings varied directly with the length of time the seeds were soaked in the Panogen-15 solution. The abnormal seedlings were characterized by the thick short roots, stunted growth of the epicotyle and brittle seedlings. The tetrazolium test was completely ineffective in estimating the toxicity caused by this chemical.

Panogen-15 injury in barley, wheat and sorghum seeds

Chemical injury with one percent Panogen-15 in small grains like barley, wheat and sorghum can

Table 13. Tetrazolium and germination test results in detecting chemical injury by Panogen-15 in corn seeds.

Treatment (40°C)		Tetrazolium test		Germination test		
Chemical	Time of soaking	% Viable	% nonviable	% normal	% abnormal	% dead
Distilled water	ten minutes	95	5	96	3	1
Panogen-15	ten minutes	93	7	73	26	1
Panogen-15	thirty minutes	93	7	8	91	1
Panogen-15	sixty minutes	92	8	9	91	0

well be identified with the tetrazolium test. The results are shown in Table 14. In the germination test of treated barley, wheat and sorghum seeds it was found that the response to the chemical was similar to that of corn. The toxicity symptoms of the chemical in the germination test was identified by thick and short club shaped growth of the radicle and very little growth of the epicotyle. With increase of the time of the treatment the toxicity was also increased in all the three crops as is shown in Table 14. In barley the tetrazolium test gave results similar to those of the germination test. In the non-treated sample 89 percent of the seeds were perfectly stained, whereas, in the treated seeds the number decreased as the period of soaking the seeds in the chemical solution has increased. Damage, in the form of necrosis, mainly in the growing points of the embryo i.e. the tip of the epicotyl, and the radicle, was observed. In wheat and sorghum the trend was similar to that of barley except for the fact that the tetrazolium test gave a higher estimate of viability when compared to the germination test.

Chemical injury by Panogen-15 in cotton seed

The tetrazolium test seemed to be ineffective in detecting chemical injury caused by the one percent solution of Panogen-15 (Table 15). High toxicity was observed by the

Table 14. Tetrazolium and germination test results in detecting chemical injury by Panogen-15 in barley, wheat and sorghum seeds.

Crop	Treatment (40°) Chemical	Time of soaking	Tetrazolium test % viable	Tetrazolium test % nonviable	Germination test % normal	Germination test % abnormal	Germination test % dead
Barley	Distilled water	five minutes	89	11	94	3	3
	Panogen-15	five minutes	85	15	94	5	1
	Panogen-15	thirty minutes	2	98	0	96	4
	Panogen-15	sixty minutes	0	100	0	75	25
Wheat	Distilled water	five minutes	91	10	91	4	5
	Panogen-15	five minutes	78	22	57	39	4
	Panogen-15	thirty minutes	35	65	0	77	23
	Panogen-15	sixty minutes	10	90	0	25	75
Sorghum	Distilled water	five minutes	79	21	78	19	3
	Panogen-15	five minutes	65	35	22	75	3
	Panogen-15	thirty minutes	28	72	0	96	4

Table 15. Tetrazolium and germination test results in detecting chemical injury by Panogen-15 in cotton seed.

Treatment (40°C)		Tetrazolium test		Germination test		
Chemical	Time of soaking	% viable	% nonviable	% normal	% abnormal	% dead
Distilled water	two hours	85	15	85	7	8
Panogen-15	two hours	80	20	1	88	11
Panogen-15	five hours	82	18	0	88	12

germination test when the samples were treated for two and five hours at 40°C. Toxicity in the germination test was recognized by short and thick root growth. Each seed showed symptoms of toxicity, and the percentages of abnormal seedlings were almost the same in the two hour and five hour treatments. At the five hour treatments, however, the toxicity symptoms were more severe. On the other hand, there was no difference between the treated and non-treated seeds in either the staining pattern or in the intensity of coloration in the tetrazolium test.

Chemical injury by Panogen-15 in vetch seed

Prominent symptoms of chemical injury by one percent Panogen-15 were also observed in vetch. The toxicity symptoms observed in the germination test were similar to those observed in other crops. Toxicity was identified by short growth of the epicotyl, and by short and knubby roots. The results of the germination test and of the tetrazolium test are given in Table 16. In the tetrazolium test of the Panogen treated material peculiar discolorations of the cotyledons may be termed as light yellow, light pink, light and deep gray, and also different combinations of these colors were observed. From the results it appears that the tetrazolium test gives a good estimation of the toxicity of the treatment to the treated seed.

Table 16. Tetrazolium and germination test results in detecting chemical injury by Panogen-15 in vetch seed.

Treatments (40°C)		Tetrazolium test		Germination test		
Chemical	Time of soaking	% viable	% nonviable	% normal	% abnormal	% dead
Distilled water	one hour	92	8	93	3	4
Panogen-15	one hour	43	57	17	78	5
Panogen-15	two hours	24	76	6	85	9
Panogen-15	five hours	7	93	0	88	12

Although the tetrazolium results appeared little higher than the actual germination test results, it should be mentioned that the intensity of coloration and the pattern of staining were different from those in the viable group of the non-treated seeds. In the tetrazolium test of the treated samples seeds which were considered viable showed a relatively lighter staining than those of the non-treated samples. This lighter staining is detectable only if all the stained seeds are placed side by side. In the non-treated sample it was observed that the staining intensity of the viable seeds was almost uniform, whereas, in the treated sample, about one half of the viable seeds showed variation in the staining intensity of the cotyledons. The treated seeds which were considered nonviable in the tetrazolium test had developed an off color of both light and deep yellow, pink, and gray, and combinations of these colors. These variations in the coloration and the intensity of the color in the nonviable seeds were very easily detectable. Delouche et al. (8) reported that one of the limitations of the tetrazolium test is that it can not detect injury caused by chemicals. The authors did not mention anything about the chemicals or type of the treatments. Cobb (6) reported the inability of this test to detect injury in seeds fumigated with methyl bromide. No work is reported

on the use of the tetrazolium test for detecting seed injury caused by liquid chemicals.

The results of this study show that the effectiveness of tetrazolium chloride in detecting chemical injury depends on the chemical itself as well as the kind of seed used. Different chemicals act differently in causing injury to seeds. Therefore, several chemicals must be tried on different kinds of seeds before any final conclusion on the use of tetrazolium chloride for detecting chemical injury can be made.

SUMMARY AND CONCLUSION

Experiments were conducted during the years 1964 and 1965 in the Seed Technology Laboratory of the American University of Beirut to study the use of the tetrazolium test in the estimation of seed viability and seed vigor. Detection of chemical toxicity caused by mercuric chloride and Panogen-15 was also included in this study. Crops used for testing viability and chemical toxicity by Panogen-15 were corn (Zea mays), sorghum (Sorghum vulgare), barley (Hordeum vulgare), wheat (Triticum spp.), cotton (Gossypium sp.) and vetch (Vincia sativa). Wheat was not used in the test of vigor, and mercuric chloride was only used on barley seed.

The results showed that good estimation of viability of seed was obtained by the tetrazolium test in all the crop seeds used in this study in that most of the samples when tested by the tetrazolium chloride gave results comparable to those of the standard official germination test.

Seed vigor was tested by the cold test in corn and sorghum. Barley, cotton and vetch seedlings were classified into vigorous and less vigorous groups on an arbitrary scale. It appeared from the tetrazolium test

results in corn, barley and sorghum samples, that seeds which were relatively more deteriorated showed a greater number of light stained seeds. It seems that on the basis of the intensity of coloration, the tetrazolium test may be used as a good estimate of vigor in graminaceous seeds. In cotton, a decrease in vigor seems to be due to the presence of necrosis on the embryonic tissue. The tetrazolium test can detect accurately the presence and extent of these necroses. The tetrazolium test failed to give a good estimate of vigor in vetch. Neither the staining intensity nor the staining patterns could be used in detecting vigor in this seed.

The tetrazolium test failed to detect chemical damage caused by mercuric chloride to barley seed. Toxicity caused by Panogen-15 in corn and cotton also was not detected by this test. However, a good estimation of toxicity by Panogen-15 in barley, wheat, sorghum and vetch was obtained. Delouche *et al.* (8) have reported as a disadvantage of the tetrazolium test that it can not detect chemical injury. It seems that further investigation with a wide range of chemicals on many crops is necessary before any conclusion can be made on the application of the tetrazolium test for detecting chemical toxicity in seeds.

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APPENDIX

Tolerances are latitudes of variation between test results. There are different tolerances recognized by the International Seed Testing Association. These are tolerances for purity, for weed and crop seeds in a unit weight, for volume weight determinations and for germination. The following germination tolerances are recognized between two germination tests:

Table 17. Table of tolerances (1).

Range of germination (percent)	Allowed tolerance (percent)
97 -100	4
95 - 96	5
90 - 94	6
80 - 89	7
70 - 79	8
60 - 69	9
Below 60	10