Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
www.phytojournal.com
JPP 2020; 9(2): 87-93
Received: 16-01-2020
Accepted: 18-02-2020

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# Standardization on seed testing protocols and seed quality parameters in medicinal plants *Embelia tsjeriam-cottam* and *Trichosanthes tricuspidata*

**Ranber Chauhan, Sobha and JS Chauhan**

**Abstract**
Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific parts of plant. Based on experimental result 1% and 5% concentration of TZ salt with 18 and 24-hour incubation period shows high colour intensity of foramen. 92% seed viability shown in seeds of *Trichosanthus.*98 % seed viability shown in seed of *Embelia*. Colour intensity of foramen on Embelia seeds was high, that treated with 1%, and 0.5% TZ solution for 18 and 24 hour incubation period. Best treatment for viability test for Indriyas in 1% TZ solution with 18-hour incubation period with 30-35 °C temperature. 24-hour fan dried seeds reduced their moisture content up 17.59% while one day sun dried and three day sun dried seeds reduced their moisture content up to 16.07% and 14.22% respectively. Fresh seed moisture content of *Trichosanthes tricuspidata* data was 16.67%. Dried seeds by silica gel reduced seed moisture content up to 9.01%, while 7 days sun dried and 15 days natural air dried seed lots reduced their moisture content up to 8.02 and 10.5 respectively. Thousand seed weight of Indriyas is 234.8gm, whereas 1000 seed weight of barbering 6.048gm. 1000 seed weight is helpful for determination of submitted and working seed sample size from a seed lot.

**Keywords:** Testing protocols, *Embelia tsjeriam-cottam*, *Trichosanthes tricuspidata*

## Introduction
Medicinal and aromatic plants are economically very important, which represent significant sources of economic revenue and foreign exchange and they are considered amongst the most important agricultural products in India. Today many medicinal plants face extinction or severe genetic loss. For most of the endangered medicinal plant species no conservation action has been taken. For example, there is very little material of them in gene banks. Also, too much emphasis has been put on the potential for discovering new wonder drugs, and too little on the many problems involved in the use of traditional medicines by local communities. Medicinal plants offer alternative remedies with tremendous opportunities to generate income, employment and foreign exchange for developing countries (Rawat and Uniyal, 2004) [15]. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent cure several human diseases (Dhar *et al.,* 1999; Savithramma and Sudrasanamma, 2006) [2, 17]. Consumption of herbal medicines is widespread and increasing in recent years and nearly 80% population of developing countries relies on traditional system of medicine (WHO, 2005) [20]. *Embelia tsjeriam-cottam* (vaya-vidang) is a rambling tree with dark-brown bark. Flowers are 3-4mm small, pale green in colour tinged with purple in the centre, and drupaceous 1-seeded fruits are bright red. Flowering occurs during July to September while fruiting takes place during December to February. Fruits are used as laxative in colic, sometimes anthelmintic, traditionally with the name of 'Bayabirang' while flowers are useful in apiculture as bee-forage. The fruits of *E. tsjeriam* are used to treat worm infestation, anemia, edema, ringworm and other skin diseases, fever, anorexia, and urinary calculi.
*Trichosanthes tricuspid data* (LalIndrayan) is a large vigorous climber with tendrils. Its large white flowers have beautiful hair-like fringed petals. Its flowers occurs between March to April and fruits during June to August. *T. tricuspi data* is very important medicinal plant in several traditional systems. Its fruits and root are used in curing bronchitis, asthma, diabetes; root juice used as an emetic and seed paste applied externally on hoops and mouth disease of cattle.

This threatened medicinal plant valued for its thermo genic, carminative, depurative and laxative property. E. ribes is now reported as vulnerable due to over exploitation. The main threat of this plant is its unsustainable and indiscriminate harvesting for commercial purposes. The regeneration from seedling of this plant is very poor. Traditional propagation techniques are not successful in large scale production of this valuable species. Only accessible way for the conservation of this species is vegetative propagation through stem cutting.

Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Successful growth and establishment of plants considerably depends on optimum germination (Gorai and Neffati, 2007) [4]. Several environmental factors such as temperature, salinity, light, water availability, soil nutrient, and soil moisture simultaneously influence the germination (Tlig *et al.*, 2008) [19] Moisture content of the seed is one of the most important factor influencing the seed vigor and viability. In an organized seed programme, the measurement of moisture content determines the decision-making policies pertaining to the harvesting, threshing, processing and marketing of seeds. It is therefore, essential that moisture content should be in accordance to the requirement of seed storage behavior and desiccation and chilling sensitivity/tolerance of a species (Parihar *et al.*, 2013) [12].

Seed dormancy is one of important limiting factor in exploitation economically product of valuable specious (Gupta *et al.*, 2011) [7]. Uniform seed dormancy is created by different treatments of seed dormancy (Yucel and Yilmaz, 2009) [21]. Germination commences with the uptake of water by imbibition of the dry seeds, followed by embryo expansion (Kucera*et al.*, 2005) [10].

## Material and Method

### Study Area

The Himalaya covers 18% of the Indian sub-continent, accounts for more than 50% of Indian forest, and contains 40% of India's endemic species. The Indian Himalayan Region (IHR) is a rich reservoir of biological diversity in the world. Over 1748 species of medicinal and aromatic plants (MAPs) reported from IHR are used in different systems of medicine. Jaunsar Bawar is a cultural and geographic unit of Uttarakhand, a Himalayan state of India. Earlier regarded as Chakrata Forest Division, it now comprises three Tehsils (revenue sectors) viz. Kalsi, Chakrata and Tunei. It is situated between 30º 26´ and 31º 2´ N latitudes and 77º 38´ and 78º 4´ E longitudes. It lies between Lesser Himalayan ranges of the western Himalaya with elevation range between c.500 m to c.3071m.

**A:** *Embelia tsjeriam-cottom*

**B:** *Trichosanthes tricuspidata*

### Source of seeds

The species of *embelia tsjeriam* has been collected from Lakhamandal-Goraghati valley, situtated at Dehradun District of Uttarakhand, India. The people of this valley called jaunsari belonging to schedule tribes (ST). Jaunsari peoples were used new top bunches of Emelia Tsjeriam-cottem for vagi table and bark were used for making alcohol in their daily life from very long time. *Embelia Tsjeriam-cottom* has one seeded fruit. The ripened pulpy fruits of Emelia Tsjeriam-cottem were collected in the month between Novembers to December. Seeds were extracted from fruits by using fermentation process. Seeds of both plants were dried in shadow for pre drying. Fresh and healthy seed were used for experimental work.

### Measurement of seed length, seed width, volume and Seed density

Measurement of seed size and shape and their correlation and relationship are more important in breeding for seed yield. Seed morphology is useful for genotype discrimination. Seeds of *Embelia Tsjeriam-cottom*are black and rounded in shape. *Trichosanthus tricuspid data* seeds are oval and brownish. Vernier calipers, scale and forceps used for determination of seed length and seed width. 5ml kerosene oil poured in graduated measuring cylinder, poured seed in random quantity, and note the level of kerosene for determination of volume of seed. Seed density calculated by using formula under:

$$\text{Seed density} = \frac{\text{weight of seed}}{\text{Volume of seed}}$$

Standardization of sample size, seed testing protocol (viz. requirement of substrate, temperature, test duration and pre-treatments) and viability test (Topographical Tetrazolium Chloride test) in *Embelia Jairam -cottom* and *Trichosan thustricusp data.*

### Experimental details

### Measurement the seed length, seed width, volume and Seed density

Measure of seed size and shape and their correlation and relationship are more important in breeding for seed yield. Seed morphology is useful for genotype discrimination. Seeds of *Embelia Tsjeriam -cottom*are black and rounded in shape. *Trichosanthus tricuspid data* seeds are oval and brownish. Verniar calipers, scale and forceps used for determination of seed length and seed width. 5ml kerosene oil poured in

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**Water imbibition rate of *Trichosanthus tricuspid data* seeds**

Water absorption is the first step of seed germination. Determine seed imbibition's rate of *Trichosanthus tricuspid data* seeds by use the mass of seeds. Twelve replicates of seeds with two seeds in each replicate used. Measured fresh weight of individual seeds of each replicates and poured separately each sample in 50ml water on incubated temperature 30ºC for imbibition's period 1,2,3,4,5,22,23, and 24 hour. Now imbibed seeds sat on a paper for removed surface water and weighted by measuring machine. Determine the moisture content of 24 hour incubated seeds. Calculate imbibition's rate by formula as under

$$\text{Imbibitions rate} = \frac{\text{Imbibed seed weight} - \text{fresh weight}}{\text{Imbibed seeds weight}} \times 100$$

**Determination of 1000 seed weight**

Thousand seed weight was calculated by following the standard procedure of ISTA, 2009. Where, seed weight was determined in eight replicates of 100 seeds each. For this, eight replicates of 100 pure seeds each from the working sample counted. Each replicate is weighed in grams to the same number of decimal place as in physical purity analysis. Seeds are counted from the entire pure seed fraction. Variance, standard deviation and coefficient of variation were calculated using the falling formulae Agrawal and Singh.

$$\text{Variance} = \frac{(\sum X2) - (\sum X)\,2}{n(n-1)}$$

**Where**

X= Weight of each replicate in grams. N = number of replicates. ∑ = summation.

$$\text{Standard deviation(s)} = \sqrt{\text{Variance}}$$

$$\text{Coefficient of variation} = \frac{\text{S.D.x } 100}{\text{x}}$$

**Where**

S.D. = standard deviation

X = mean weight of 100 seed

**Standardized the submitted and working seed sample size for physical purity and seed moisture content.**

The seed sample size standard, for determination of physical purity, seed moisture content, seed viability and seed germination. ISTA has standardized the submitted and working seed sample size of many medicinal plants (International rule for seed testing, 2019). However, not described any seed sample size of *Embelia Tsjeriam -cottom* and *Trichosanthes tricuspi data*.

**Standardized the submitted and working seed sample size for physical purity**

The required quantity of the seeds which is sent to the seed laboratory for seed testing called submitted sample. Submitted sample obtained from the composite sample, it is 10 times less than the composite sample. Working sample presents as the testing sample, which used for seed certification standards, and it obtained from submitted sample. Submitted and working seed sample are depends on seed size and thousand seed weight. Used born or seed divider, seed counter board, and hand lance for obtaining submitted sample and working sample in the lab of seed science and technology. Following ISTA guidelines given in ISTA Handbook, 2003. *Embelia Tsjeriam -cottom* belongs to family of myrsinaceae and its thousand seed weight is 6.04gm. While thousand seed weight of *Trichosanthes tricuspi data* is 234.80gm. To prepare submitted sample, composite sample mixed thoroughly and reduced up to required quantity with the help of burner divider. Submitted sample is ten times more from the working sample for physical purity analysis, each working sample consists at least 2500 seeds.

**Standardized the submitted and working seed sample size for seed moisture content**

Seed moisture content of seeds is loss the moisture content and loss the weight when seed dried accordance with rule. It expressed as percentage of weight of the original sample. Submitted sample for moisture content is 100gm of the species that has to be ground and 50gm of other species (ISTA Handbook, 2003). Seeds of *Embelia Tsjeriam -cottom* and *Trichosanthes tricuspid data* have obligatory for grinding. Working sample derived from submitted sample. The moisture content determination carried out in duplicate on two independently drawn working samples each of the following weight, depending on the diameter of the containers used.

ISTA has prescribed standard for working sample are:

**If the containers are less than 8cm diameter- 4-5gm seeds for a working sample**

**If containers are 8cm diameter or larger - 10gm seeds for a working sample**

Tightly feted Containers used for working sample of *Embelia Tsjeriam-cottom*is less than 8cm diameter, while tightly feted container larger to 8cm diameter used for *Trichosanthes tricuspid data* seeds.

**Determination of seed moisture content**

So many factors affect the seeds viability and health during storage period and Seed moisture content is the most important one. Seed moisture content is the physiological parameter of seeds with low moisture content can be stored long period. Based on seed moisture content seeds classified in orthodox, semi orthodox and recalcitrant seeds.

Various equipments used in moisture test: thermostatic oven, desiccators, analytical balance, grinding machine, airtight container.

Working sample: pre dried submitted sample of 100gm of both medicinal plants derived from composite samples. Two duplicate working sample of both medicinal plants derived from submitted sample. 0.5gm working sample of *Embelia Tsjeriam -cottom* and 12gm working sample of *Trichosanthes tricuspi data* was weighted by analytic balance. Seeds of *Trichosanthes tricuspid data* were courts grounded by grinding mill. Grounded seeds weighted again and placed in oven at 117 ºC for 4 hour. At the end of the prescribed period, dried seeds placed in desiccators for 30 minutes. Dried seeds weighted with container.

**The moisture content percentage calculated by following formula**

$$\frac{\text{weight in gm before drying(M2)} - \text{weightin gm after drying(M3)}}{\text{weight in gm before drying}(M2 - M1)} \times 100 = \text{moisture(\%)}$$

**Where**

M1 = weight of empty container with its cover

M2 = weight of container with its cover and seeds before drying

M3= weight of container with its cover and seeds after drying

**Viability estimation of seeds**

The TZ method is used for viability estimation of seeds. Equipments used for conducting TZ test i.e. Petri dishes, watch glasses, beakers, blades, needles, forceps, filter paper, incubator, and magnifying devices. Prepare three concentration of Tetrazolium chloride solution i.e. 0.25%, 0.5%, and 1%, by using 1.0, 0.5 and 0.25gm TZ salt and 100ml distilled water to make 100ml TZ solution. Buffer tablets used for maintain ph 6.5 to 7.5 for the proper staining.

The working seed sample derived from submitted sample by using borner divider. Four seed replicates of both medicinal plants used and 100 seeds consist in each replicates. Seeds of *Embelia Jairam -cottom* piercing throw needle and cut the seed coat of *Trichosanthes tricuspi data* to ensure adequate penetration of solution for proper staining**.** One incubation temperature, 30 ˚C used for incubation of seed replicates. Seeds placed in solution and held in 30 ˚C temperature in dark for 6, 12, 18 and 24hr respectively. After a period in the solution, seeds removed from the solution, rinsed 2-3 times in water, and then placed in a paper to evaluate by using magnifying devices. Individual seeds evaluated as viable, non-viable based on their staining pattern. Colour intensity of foramen shown seed vigour.

**Results**

**Measurement the seed length, seed width, volume and Seed density**

Seeds of barbering are very small as compared to Indriyas and there is a big difference in shape size, density and seed weight. 1000 seed weight of barbering is just 6.048gm while Indriyas seeds contain 234.8gm seed weight. Pericarp of Indriyas seeds has hard and it is non-endospermic seed contain large cotyledon. Seeds of barbering are endospermic with smooth pericarp, round in shape and size of seeds is 2.0mm.

**Water imbibition rate of *Trichosanthus tricuspi data* seeds**.

Seed imbibition process is very quick and simple method to know about the seed coat is permeable for water or not. Hard seed can cause the physical dormancy for seeds. Therefore, it is very important to know about seed coat weather it is permeable to water. Seed coat of in drayan is hard, According to seed imbibition method seeds of indrayan permeable to water. According to table number 2, seeds gain their weight after imbibition up to 0,829. Seeds of in drayan gain their moisture content 30% after 24 hour of imbibition process. While fresh weight of seeds was just 0.579 and seed, moisture content was just 8.02%. Seed moisture content of seeds increase continues in per hour (table no.2). Seed imbibition test on the in drayan seeds determine that seeds of in drayan is not physically dormant. Seed coat of seeds is hard and tough but seed take water content by chalazal end of seeds.

Seed density calculated by using formula under seed measurement viz; seed length and width, seed density, 1000 seed weight are given in table no.1

**Table 1:** Morphological characteristics of *Trichosanthes tricuspi data* and *Embelia Tsjeriam-cottam*

| **Parameters** | ***Trichosanthes tricuspidata* (Indrayan)** | **(Baybirang)** |
|---|---|---|
| Family | Cucurbitaceae | Myrsinaceae/Primulaceae |
| Habit | Succulent herbs | shrubs |
| Seeds | Non-endospermic | endospermic |
| Seed length | 14mm | 2.08 mm |
| Seed width | 8mm | 1.90 mm |
| Seeds shape | Oblong | Round |
| Seed density | 0.883 g/cm$^2$ | 0.667 g/cm$^2$ |
| 1000 seed weight | 234.8 | 6.048 |

**Table 2:** Lakhamandal

| **Seed imbibition rate of *Trichosanthes tricuspi data*** | | |
|---|---|---|
| **Parameter** | **Seed weight** | **Seed Moisture content** |
| Fresh seeds | 0.579 | 08.02 |
| 1 hour imbibed seeds | 0.648 | 10.64 |
| 2 hour imbibed seeds | 0.667 | 13.19 |
| 3 hour imbibed seeds | 0.682 | 15.10 |
| 4 hour imbibed seeds | 0.694 | 16.57 |
| 5 hour imbibed seeds | 0.825 | 17.64 |
| 22 hour imbibed seeds | 0.766 | 29.870 |
| 23 hour imbibed seeds | 0.827 | 30.01 |
| 24 hour imbibed seeds | 0.829 | 30.16 |

**Standardization of seed sample size: standardized**

The seed sample size of both medicinal plants by using seed protocols of International seed testing association (ISTA). A Seed sample size represents the quality of seed lot. Seed sample size of both medicinal plants for physical purity test and seed moisture content test has given in table no.3. Submitted and working seed sample size of Indriya seeds for physical purity was considered 1000 grams and 587 grams respectively. For seed moisture content determination test submitted seed sample size and working seed sample size of Indriya seeds determined 100gm and 12±1gm respectively.

**Table 3:** Standardized seed sample size

| Seed test | *Trichosanthes tricuspi data* | | *Embelia Tsjeriam-cottam* | |
|---|---|---|---|---|
| | Submitted sample | Working sample | Submitted sample | Working sample |
| Physical purity test | 1000gm | 2500 seeds or 587.2gm | 60gm | 2500 seeds or 6.048gm |

Submitted seed sample size of bay baring seeds for physical purity analysis is 60 grams and for working sample it consists of 2500 seeds or 6.048 gram. While seed sample size for moisture content is 100 gram for submitted seed sample, and 4.5±0.5gm for working seed sample.

**Determination of seed moisture content**

Fresh seeds moisture content before pre-drying and seed moisture content after completion of seed drying process are given in table no.4. Fresh seed moisture content of bay birang is 36.92 percent. 24-hour fan dried seeds reduced their moisture content up 17.59% while one day sun dried and three day sun dried seeds reduced their moisture content up to 16.07% and 14.22% respectively. Fresh seed moisture content of *Trichosanthus tricuspi data* was 16.67%.dried seeds by silica gel reduced seed moisture content up to 9.01%, while 7 days sun dried and 15 days natural air dried seed lots reduced their moisture content up to 8.02 and 10.5 respectively.

**Table 4:** Seed moisture content of *Embelia tsjeriam-cottam and Trichosanthes tricuspi data at different time interval.*

| **Seed moisture content of *Embelia Tsjeriam-cottam*** | | | |
|---|---|---|---|
| Fresh seed | 24 hour | One day sun dried | Three day sun dried |
| 36.92 | 17.59 | 16.90 | 15.22 |
| **Seed moisture content of *Trichosanthes tricuspi data*** | | | |
| Fresh seeds | 7 days sun dried | 12 days silica gel dried | 15 days natural air dried |
| 16.67 | 8.02 | 9.01 | 10.5 |

**Standardize the seed viability test (2, 4-D Tetrazolium chloride test)**

Three concentration of TZ solution with for incubation period on 30°C temperature was given for the experiment on seed viability test for both plants. Stained parts of both plant species, evaluated manually by using magnifying device. Individual seeds were evaluated and stained seed parts viz; embryo, endosperm, or cotyledons recognized as viable seeds. Unstained embryo with endosperm or cotyledon considered dead seeds. All results related to this experiment are given in table no.5.

**Table 5:** Colour intensity of for mazan in seeds of Embelia tsjeriam-cottam and Trichosanthes tricuspi data at different concentration of TZ salt.

| Concentration of TZ | Incubation period | Colour intensity (baybidang) | Colour intensity (Indrayan) |
|---|---|---|---|
| 1% | 6 | low | Low |
| | 12 | medium | medium |
| | 18 | high | high |
| | 24 | high | high |
| 0.5% | 6 | low | Low |
| | 12 | medium | medium |
| | 18 | medium | high |
| | 24 | high | high |
| 0.25% | 6 | low | Low |
| | 12 | low | Low |
| | 18 | medium | medium |
| | 24 | high | high |
| Viability % | | 95% | 92% |

Based on experimental result 1% and 5% concentration of TZ salt with 18 and 24-hour incubation period shows high colour intensity of foramen. Colour intensity of foramen (red colour) on seeds is most important factor to standardized concentration of TZ solution, incubation period, and incubation temperature seed vigour and seed viability. 92 percent seed viability shown in seeds of *Trichosanthus.*98 percent seed viability shown in seed of *Embelia*. Colour intensity of foramen on *Embelia* seeds shown high, that treated with 1%, and 0.5% TZ solution for 18 and 24 hour incubation period. Six-hour incubation period shown low intensity of colour in all concentration of TZ solution. Best treatment for viability test for in drayan in 1% TZ solution with 18-hour incubation period with 30-35°C temperature. Seed coat breaked is obligatory for seed viability test of Trichosanthes tricuspid data, while Emelia seeds piercing with needle are obligatory for penetration of TZ solution.

**Discussion**

According to the world health organization, 80 percent of the population is associated with traditional medicine. At present, there are more possibilities to cultivate medicinal and aromatic plants because the production of these plants can be easily taken up in the climate of India. In medicinal crops, not much work has been done on the seed technological aspects especially the seed testing procedures and seed storage techniques. The information available on these aspects is scanty.

For a successful crop production, the use of good quality seed is very essential which increase the yield by 20-25%. The extent of this increase is directly proportional to the quality of seed that is being sown. Seed size is one of the components of seed quality, which affects the performance of crop (Adebisi *et al.*, 2011) [1]. Size is a widely accepted measure of seed quality and large seeds have high seeding survival growth and establishment (Jerlin and Vadivelu 2004) [9]. A wide array of different effects of seed has been reported for seed germination, emergence and related agnominal aspects in many crop species. Generally, large seed has better field performance then small seed. Germination rate and seedling vigour index values increased with the increase of seed size suggesting the selection of larger seeds for good stand establishment (Roy *et al.*, 1996) [16].

Measurement of seed size and shape and their correlation and relationship are more important in breeding for seed yield. Seed morphology is useful for genotype. Seed size of Indrayan is 14×8mm, while seed size of baybidang is just 2.08×1.90 mm. Morphological characteristics of seed shown soundness of seed. This is the first visual method to know seed health and vigor. Thousand seed weight of in drayan is 234.8gm, whereas 1000 seed weight of bay birang 6.048gm.

1000 seed weight is helpful for determination of submitted and working seed sample size from seed lot.

Seed imbibition process is very quick and simple method to know about the seed coat is permeable for water or not. Hard seed can cause the physical dormancy for seeds. Seed imbibition test on the in drayan seeds determine that seeds of in drayan is not physically dormant. Seed coat of in drayan is hard and tough but seed take water content by chalazas end of seeds. Based on experiment result 1%, 5%, and 0.25% concentration of TZ salt with 6, 12, 18 and 24hour incubation period shows different colour intensity of for mazen. Colour intensity of for mazen (red colour) on seeds significantly affect factors that concentration of TZ solution, incubation period, incubation temperature seed vigour and seed viability. On bay birng seeds treated with 0.25% TZ solution shown low intensity after6 and 12 hour incubation period, while at 18 and 24 hour incubation period, seed sown medium colour intensity in both medicinal plants. Seeds treated with 0.5% TZ solution seed colour intensity at incubation period of 6 and 12 hour shown low and medium respectively in both medicinal plants seeds, whereas on same concentration (0.5%) of TZ at 18 hour incubation period shown medium colour intensity of baybidang seeds and high colour intensity shown on indrayan seeds. Both plants seeds treated with 0.5% TZ solution on 30°C incubation temperature with 24 hour incubation period shown high colour intensity. Seeds treated with 1% TZ solution for 6 and 12-hour incubation periods at constant temperature (30 °C) seeds shown low and medium colour intensity, whereas with 18 and 24 hour incubation period son same concentration seed shown high colour intensity. 92 percent seed viability shown in seeds of *Trichosanthus.*98 percent seed viability shown in seed of *Embelia*. Seed coat breaked is obligatory for seed viability test of Trichosan thus tricuspi data, while Embelia seeds piercing with needle are obligatory for penetration of TZ solution.

Seed sample represent the seed lot. It is used in seed testing laboratory to find out the information about seed lot viz' genetic purity of seed lot, physical purity of seed lot, quality and health of seed lot, moisture content, longevity and viability of seed lot. Submitted sample of in drayan for physical purity and seed moisture content test is 1000 gram and 100 gram respectively. Working seed sample of in drayan for the same test is 2500 seeds or 587.2gm and 12±1gm (Table no.3). Submitted and working seed sample size of bay birang for physical purity test is 60gm and 2500 seeds or 6.048gm, while for determination of seed moisture content submitted and working sample has standardized 100gm and 4.5±0.5gm. A perfect amount of seed sample is depends on seed size shape and 1000 seed weight. Seed sample size of many crops has find out by ISTA. However, ISTA did not standardized minimum seed certification standards and seeds sample size of two important medicinal plant, *Embelia tsjeriam-cottam* and *Trichosanthes tricuspidata*. Follow ISTA guidelines for standardization of seed sample size of both medicinal plant species. Since there was no information available regarding tetrazolium staining procedures of *Embelia tsjeriam-cottam and Trichosanthes tricuspidata* in 'Tetrazolium Testing Handbook' of AOSA.

## Conclusion

The present study filled the gap by standardizing the procedures such as method of pre conditioning, excision technique, concentration of staining solution, incubation time and temperature and finally method of evaluation. Based on experimental result 1% and 5% concentrations of TZ salt with 18 and 24-hour incubation period shows the highest colour intensity of formazen. Colour intensity of formazen (red colour) on seeds is most important factor to standardized concentration of TZ solution, incubation period, and incubation temperature seed vigour and seed viability. Colour intensity of formazen on *Embelia* seeds shows high, that treated with 1% and 0.5% TZ solution for 18 and 24 hour incubation period.

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