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Research Paper

Optimization of Seed Germination of *Berrya cordifolia* by Novel Seed Treatment Techniques

Senanayaka S.M.C. ^{11*}

¹ Department of Forest Conservation, Baththaramulla, Sri Lanka

* Corresponding Author: mareshchaturange@gmail.com  <https://orcid.org/0009-0007-0606-037X>

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Abstract: *Berrya cordifolia* is a deciduous tree that can grow up to 40 meters tall, though in some parts of its range it barely exceeds 6 meters. The bole can be 10 - 50cm in diameter and is unbranched for up to eleven meters. The tree is having fibrous bark, the showy green colour flowers are produced in large tight clusters. The tree can be found in Southeast Asia and the Pacific Region. The plants are valuable

for their timber. *Berrya cordifolia* is a valued timber species in Sri Lanka, where it is commonly exploited from the wild. The tree is being grown in plantations in several areas in the tropics. *Berrya cordifolia* is the one of major plant species propagating by seeds in forest nurseries. However, uniformity of seed germination and germination percentages of untreated *Berrya cordifolia* seeds are very low even under the normal seed treatments. Therefore, germination of *Berrya cordifolia* seeds in forest nurseries has been becomes a very costly and time-consuming task. Therefore, the present study was conducted with the objective of identifying a novel seed treatment technique to increase the efficiency of *Berrya cordifolia* seed germination, which will reduce cost and time consumption for production of *Berrya cordifolia* seedlings in nurseries. In this study, it was found that treatment in soaking the seeds in cow dung extract is the best pre sowing treatment for *Berrya cordifolia* seeds with 66.4 (± 2.61) % average germination percentage. Average germination speed was 04 days from sowing (time taken to germinate 50% of final population) and 0.6 (± 0.55) days average germination uniformity (difference between the time taken to germinate 75% of final population and time taken to germinate of 25% of final population).

Keywords: *Berrya cordifolia*, timber, seed treatments, germination percentage, germination speed, germination uniformity



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Introduction

Berrya cordifolia (Halmilla, Trincomalee wood) is a species of tree native to much of tropical Asia and introduced to Africa. It is also found in the forests of Philippines, Andaman Islands, Bangladesh, Borneo, Christmas Islands, India, Jawa, Laos, Lesser Sunda Islands, Malaya, Myanmar, Sri Lanka, Sulawesi, Taiwan, Thailand and Vietnam. *Berrya cordifolia* is

a tree 4-6(-35) m tall, 10-20 cm DBH at maturity. Stipules filiform, 1-2 cm, glabrous; petiole 3.5-10 cm; leaf blade ovate to ovate-oblong, 10-20(-25) $\times$ 6-8(-14) cm, thinly leathery, very sparsely stellate pubescent, soon glabrous, lateral veins 4 or 5 pairs, base cordate, apex acuminate. Panicles are approximately 20 cm, hairy. Pedicel is 10 mm in length, calyx 3-5-lobed, 3-5 mm, lobes triangular-ovate, and hairy. Petals are white or pink in colour, filiform, 6-8 mm, becoming reflexed. Stamens are 5-

6 mm. Ovary hairy; style short. Capsule with persistent calyx, globose, 1-1.3 cm wide, pubescent, with 6(or 8) horizontally spreading thin wings, wings $2.5-3 \times 0.7-1$ cm. Seeds are approximately 6

$\times 3$ mm, with brown or yellow, caduceus bristles (Flora of China).



Figure 1. Seeds and fruits of *Berrya cordifolia*.

Heartwood is dark red-brown, with darker streaks, distinctly demarcated from whitish to pale brown sapwood. Wood surface is dull, with slightly oily feel. Freshly sawn wood has a pungent odor. It is widely used for timber, in India and Myanmar, the wood of *Berrya cordifolia* is commercially traded. In the 1970s, annual exports from Myanmar amounted to about 500 t (600 m³). The wood has a number of uses, for furniture making and historical applications in shipbuilding. Suitable for light and heavy construction, flooring, mine props, cartwheels, tool handles, agricultural implements, poles, carving, turnery, paneling, paper making, etc. In the Philippines, used for making boats and native houses. Its bark is used for fibers; Bark yields a fiber of low quality. A hot water extract of leaves has shown antifungal activity against *Pythium aphanidermatum*. A cold-water extract showed no activity.

Seed germination

Seed germination refers to the process by which a plant embryo within a seed emerges from dormancy and begins to grow, forming a seedling. This process is influenced by a combination of internal factors within a seed and external environmental conditions. Main internal factors affecting the seed germination are seed viability, seed dormancy, seed coat dormancy, embryo dormancy, chemical inhibitors. Availability of water and oxygen, optimal temperature for germination, light availability, mother bed conditions are the major external factors affecting on the seed germination. Seeds of many tree species germinate readily when subjected to favorable conditions of moisture and temperature. However, other species possess some degree of seed dormancy. Where dormancy is strong, some form of seed pretreatment is essential in artificial regeneration, in order to obtain a reasonably high germination rate in a short time. In experiments with *Berrya cordifolia*, the treatments gave an increase in

germination percentage compared with the control. In other cases the main difference may be in the speed of germination rather than the final total, e.g. in *Pinus taeda* (Bonner *et al.*, 1974) and *P. elliottii* (Forrest, 1964). Where dormancy is slight, the effect of pretreatment may be marginal. The benefits from pretreatment (savings in seed and seed-bed space, predictable and concentrated period of transplanting, more uniform nursery stock) need to be weighed against the cost and trouble of treating. The decision whether or not to pretreat the seed will vary not only in accordance with the species but also with provenance, seed year, local nursery conditions, length, and conditions of storage.

Pretreatment to terminate dormancy and speed up germination is thus one important type of pretreatment. Another comprises various forms of seed dressing, pelleting or coating, designed to give the seed protection against pests, diseases or adverse conditions, to improve the uniformity of seeds or to render them more visible to the nurseryman.

Classification of Types of Dormancies

Dormancy may be of several different types and sometimes more than one type occurs in the same seed. The simplest grouping distinguishes between exogenous or seed coat/ pericarp dormancy, endogenous or embryo dormancy and combined dormancy, in which both seed coat and embryo dormancy occur at the same time.

More detailed classifications of dormancy have been published. That by Nikolaeva M.G., 1977 has been applied, in simplified form, to temperate broadleaved tree and shrub seeds by Gordon A.G. and Rowe D.C.F., 1982. In most species of the moist tropical forests' dormancy is not a problem. Conditions of temperature, moisture and oxygen are almost invariably suitable for germination immediately on dispersal, so most species germinate within a matter of days or weeks and would not benefit from dormancy. In the drier tropics, on the other hand, seed coat dormancy is common and some form of pretreatment is necessary to obtain rapid and uniform germination. Both physical dormancies, caused by hard seed coats or pericarps with cutinized layers, which are impermeable to water, and chemical dormancy, caused by inhibiting chemicals present in the seed

covering, occur; it is likely that they sometimes occur simultaneously in the same seed. But it is often difficult to differentiate between them, since a treatment which softens the seed coat, e.g. hot water, will automatically leach out inhibitors at the same time.

As pointed out by Gordon A.G. and Rowe D.C.F., 1982, the covering of seeds, which exhibit mechanical dormancy is thick, tough and resistant to embryo growth but, unlike the "hard seeds" characteristic of physical dormancy, it is permeable to liquids. The severe treatments, such as boiling water or acid, which can be effective in overcoming physical dormancy, would penetrate the covers of the mechanically dormant seeds and kill the embryos. Mechanical dormancy occurs in a number of temperate genera e.g. *Crataegus*, *Carpinus*, *Elaeagnus*; it is not known whether it occurs in the tropics.

In temperate forestry, methods of overcoming the various types of endogenous dormancy have been investigated for a long period and the most important treatments. It is doubtful, whether any of these methods will have application in the lowland tropics, but some of them may be applicable to species planted in the sub-tropics and tropical highlands; for example, germination of *Pinus taeda*, an important species for afforestation in some tropical highlands and in the sub-tropics is known to benefit from cold moist pretreatment (Krugman S.L. and Jenkinson J.L., 1974).

In general, however, the physical and chemical types of exogenous dormancy are likely to be of most concern to tropical foresters.

Treatments Designed to Break Exogenous or Seed coat Dormancy

Seeds of some species have hard, cutinized seed coats that completely prevent the imbibition of water and sometimes the exchange of gases. Without imbibition and gas exchange renewal of embryo growth and germination are impossible. Physical seed coat dormancy of this kind occurs most frequently in species adapted to alternating dry and wet seasons, including several leguminous genera such as *Acacia*, *Prosopis*, *Ceratonia*, *Robinia*, *Albizzia*, *Cassia*. In some species, e.g. *Tectona grandis* and *Pterocarpus angolensis*, the hard layer is formed by the pericarp or fruit. The unit of dispersal, storage and sowing in *Tectona* is the fruit, often

referred to loosely as the “seed”, but for practical purposes, the origin of the impermeable layer does not affect the choice of pretreatment.

Pretreatment to overcome physical seed coat dormancy are designed to soften, puncture, wear away or split the seed coat in order to render it permeable, without damaging the embryo and endosperm within. They include physical and biological methods, dry heating and soaking in water or chemical solutions. Any treatment, which destroys or reduces seed coat impermeability, is commonly known as scarification (Bonner F.T., 1984). Destruction of impermeability at a single point in the seed coat is normally sufficient to allow imbibition and gas exchange.

Chemical seed coat dormancy, caused by the presence of chemicals located in the seed coat but inhibiting germination of the embryo, can usually be overcome by some form of liquid treatment which leaches out the chemicals. The wet treatment methods should therefore be effective in breaking chemical, as well as physical seed coat dormancy, and a combination of the two. Dry methods on the other hand, which are effective in overcoming physical dormancy, are unlikely to have any effect on chemical dormancy.

Physical methods

One of the simplest and most direct physical methods is to cut, drill or file a small hole in the coat of each seed before sowing (Goor and Barney, 1976). This method has been found appropriate in the Philippines for large leguminous seeds in the genera *Azalia*, *Albizia*, *Intsia* and *Sindora* (Seeber G. and Agpaoa A., 1976) and in Honduras for *Acacia*, *Prosopis*, *Enterolobium* and other legumes (Robbins A.M.J., 1982). *Intsia* seed is nicked at each end and a third time in the area of the hilum and micropyle; the last area is the most important. In Tanzania the hard and bristly pericarp of *Pterocarpus angolensis* is chipped at one edge by knife (Laurie M.V., 1974) or cracked by beating with clubs (Boaler S.B., 1966). In the Philippines the hard seed coat of *Eusideroxylon* is cracked by hammer. In *Calophyllum* complete removal of the seed coat has produced better germination than nicking (Seeber G. and Agpaoa A., 1976). Sand-paper may also be used to reduce seed coat thickness by abrasion. In trials in Pakistan sandpaper proved the most effective treatment in increasing and accelerating germination in a number of hard-

coated species (Nisa S.H. and Qadir S.A., 1969). As an example, *Leucaena* seeds had zero germination in the untreated control and after 24 hours' soaking in cold water. This was increased to 42 % in 26 days by 1 minute soaking in concentrated H_2SO_4 , to 60 % in 13 days by 2 minutes in boiling water, and to 100% in 3 days by sandpaper treatment. In laboratory trials in Sweden, sandpaper scarification followed by a three hour cold water soak, was the most effective treatment for *Acacia farnesiana*, yielding 88 % germination in 7 days and 100 % in 21 days, compared with 63 %, 23 % and 3 % in 21 days, respectively for soaking in concentrated sulphuric acid, absolute alcohol and hot water. In six other hard-seeded species from Iraq tested at the same time, it was less effective than one or other of the soaking treatments (Kisou J. et al., 1983). Manual treatment of individual seeds is slow but, with experienced workers, is safe and effective. It is best suited to the larger and more refractory seeds e.g. *Delonix regia* (Wunder W.G., 1966). The effect of physical scarification may be reinforced by soaking in cold water before sowing.

For treatment of large quantities of seed, mechanical scarification is more suitable than the manual method. Seed may be tumbled or churned in a concrete mixer with sharp gravel or sand, or in a special drum lined with abrasive material such as sandpaper, cement or crushed glass or incorporating rotating abrasive disks (Kemp R.H., 1975, Goor A.Y. and Barney C.W., 1976). If gravel or sand is used, it should be sieved to ensure that it can be readily separated from the seed by use of an appropriate mesh screen. The method is not suitable for seeds with abundant resin or pulp, which may clog the machine. Care must be taken to avoid overtreatment causing damage that can reduce or destroy germinability of the seeds. Examination of the seed coat surface, if necessary by hand lens, or testing the capacity of the seed to take up water, demonstrated by swelling, can be used to estimate effectiveness of scarification (Bonner F.T. et al., 1974).

In India, mechanical scarification is used successfully to overcome seed coat dormancy in *Acacia catechu*, *A. nilotica* subsp. *indica*, *Albizia falcata*, *A. lebbek*, *Cassia fistula*, *C. javanica*, *C. nodosa*, *Delonix regia*, *Dichrostachys cinerea*, *Santalum album*, *Terminalia arjuna* and *T. tomentosa* (Pattanath P.G., 1982). Horizontal shaking of seeds of *Acacia farnesiana*, bottled with crushed glass, for five

hours gave a germination of 20.7 % plus additional imbibed seeds 10.7 % in 43 days, compared with 0.7 % + 0.7 % for the control in the same period. But this result was much inferior to that obtained with the same species by manual scarification with sandpaper, noted on p. 167 (Kisou J. *et al.*, 1983).

Soaking in water

A number of treatments involve soaking seeds in water or other liquids. These wet treatments may combine the effects of softening hard seed coats and leaching out chemical inhibitors.

Some seeds, which have little resistance to germination may respond well to soaking for 24 hours in water at ambient temperature (Kemp R.H., 1975). This may be the result of a more rapid imbibition than can be achieved in a moistened nursery bed. It is recommended for some species after manual, mechanical or acid scarification (Seeber G. and Agpaoa A., 1976, Elamin H.M., 1975, Matias A.R. *et al.*, 1973) found that 48 hours' soaking of *Pinus caribaea* seed in water at room temperature gave more uniform germination than in the untreated seed.

In India soaking in water, for periods varying from two to 48 hours according to species, accelerates germination in *Acacia mearnsii*, *A. melanoxylon*, *A. nilotica* subsp. *kraussiana*, *Adenanthera microsperma*, *Albizia amara*, *A. procera*, *Grevillea robusta* and *Trewia nudiflora* (Pattanath P.G., 1982).

A more effective treatment, especially in hot climates, is alternate wetting and drying of seed. This has frequently been used for *Tectona* fruits. In Thailand, after scarification, alternate soaking and drying was applied, 4 times soaking and 3 times drying, each for 30 – 45 minutes (Bryndum K., 1966). Elsewhere, if preliminary scarification is not applied, common practice is to spread the "seed" out on a hard surface in the sun in a layer about 5 cm deep and to soak it thoroughly; then to turn it over from time to time, allowing it to dry out and bake in the sun for a day or two. This process of alternate soaking, drying and baking is repeated a number of times, usually between five and ten cycles, until signs of germination appear. Each cycle of wetting and drying may be one day for soaking and 3 – 5 days for drying and baking. As soon as germination starts, the "seed" should be sown in the nursery (Laurie M.V., 1974). In Tanzania, the

preliminary soaking is for 72 hours in full sacks in a stream or in large drums. It is then sown on the surface at the rate of 5 kg/m² and, after two days in the sun, is covered with about 2 – 5 cm depth of soil and watered daily (Wood P.J., 1967).

Tectona grandis is one tropical species in which there is evidence that chemical dormancy caused by inhibitors in the pericarp may be more important than physical dormancy. Fairlamb J. and Davidson J., 1976 found that an aqueous extract, obtained by soaking *T. grandis* fruits for 4 days and used to moisten filter paper, inhibited germination of cress seeds. Germination was 11 % in 144 hours in the extract, compared with 76 % in tank water and 96 % in distilled water. On the other hand, Pattanath P.G. (1982) could find no evidence for physical dormancy in *T. grandis*. She found that, after soaking the fruits in water for 24 hours, water had soaked right through to the locules where the seeds were attached. There is great variation in the degree of dormancy between provenances of teak.

Some require no pretreatment, some require alternate wetting and drying as described above, others respond to soaking for four hours in Sach's nutrient solution, which may indicate a nutrient imbalance in the seeds (Gupta B.N. *et al.*, 1975).

Chemical dormancy apparently occurs also in *Terminalia ivorensis*. Alternate daily soaking and drying of the fruits of this species for seven days was effective in producing 50 – 70 % germination, whereas germination of untreated seed is usually poor (Brookman-Amissah J., 1973). In a later experiment the same author found that an aqueous extract from *Terminalia ivorensis* fruits had a slight inhibitory effect on germination of lettuce seeds but a more serious effect in the number of malformations in the germinated seedlings. He identified Coumarin as one of the more important inhibitors involved (Brookman-Amissah J., 1976). Soaking in running water for one to two weeks has been used successfully to remove inhibitors in *Atriplex* spp. (Turnbull J.W., 1983).

Hot water treatment has given good results with a number of leguminous seeds. The seeds are usually placed into boiling water, which is immediately removed from the heat source and left to cool gradually, the seeds remaining in the water for about 12 hours (Kemp R.H., 1975). They imbibe and swell as the water cools. The proper relationship of the volume of water to volume of seeds can be determined by experiment. It may vary considerably according to species, ratios of 2 – 3

times (Goor A.Y. and Barney C.W., 1976), 4 – 5 times (Bonner F.T. *et al.*, 1974) and 5 –10 times (Seeber G. and Agpaoa A., 1976) as much water as seed have been suggested. Some species respond better to an initial temperature well below boiling e.g. *Albizzia falcataria* (Valencia A.R., 1973). An initial temperature of 90° C, cooled to ambient 20° C, has given good results with *Parkinsonia aculeata* and *Ziziphus spina-christi* (Kisou J. *et al.*, 1983). Of various initial water temperatures and periods of soaking and cooling tested for *Leucaena leucocephala* in the Philippines, one minute soaking from an initial temperature of 80° C gave the best result 90 % germination (Alvarez-Racelis E. and Bagaloyos A.P., 1977). The period of soaking and cooling appeared to have little effect, e.g. an initial 80° C and 6 hours' soak and cool gave 89 % germination. The initial water temperature had a big effect; germination was only about 30 % after an initial temperature of 100° C and only about 25 % after an initial 40° C.

Bowen M.R. and Eusebio T.V., 1981 b found that in *Acacia mangium* seeds in Sabah there was a close correlation between the initial temperature of the water and the subsequent germination. Germination increased progressively from 5 % after immersion in water at 30 °C to 91 % after immersion at 100° C. Prescribed treatment for this species is now to immerse seeds in 5 x their volume of water at 100° C, stir in the cooling water for 30 seconds, pour off hot water and soak overnight in 20 x their volume at room temperature. For *Albizzia falcataria* a lower initial temperature of 80°–90° C is recommended (Bowen M.R. and Eusebio T.V., 1981a). Some species of *Acacia* require more severe treatment. Larsen E., 1964 found that, whereas the standard treatment with boiling water improved germination of *Acacia sieberiana*, an exceptionally resistant species, from 2 % to 10 %, continuous boiling of the seed in water for 60 minutes achieved 60 % germination within two weeks.

Prescriptions for hot water treatment must be applied meticulously if seed coat dormancy is to be removed without killing the seeds through excessive heating. It is often easier to ensure this at a central research laboratory than in a number of scattered field nurseries. In Sabah it was found possible to redry seeds of *Acacia mangium* and *Albizzia falcataria* after the initial 30 seconds' hot water treatment, package and dispatch them to field nurseries and, after 3 days' storage, to give a simple cold water soak before sowing. Germination was just as good (c. 80%) as when seeds were sown

immediately after treatment (Bowen M.R. and Eusebio T.V., 1982). Drying was done in a current of dry air at 45°C for periods of 6 – 18 hours.

Hot water treatment is relatively easy and safe to apply, as well as being effective with some species. It is not well adapted to large lots because of the difficulty in handling and sowing the swollen seeds (Heit C.E., 1967 b).

Acid treatment

The chemical most commonly used to break seed coat dormancy is concentrated sulfuric acid. For some species, it is more effective than hot water treatment. Seed, which has been kept for a long period in store, may require a longer period in the acid than fresh seed, which could be severely damaged by the same length of treatment (Kemp R.H., 1975c). Great care is needed in the handling of sulfuric acid and this method is not suitable for use by unskilled workers.

Bonner F.T. *et al.*, 1974, give detailed instructions for use of sulfuric acid.

The objective of present study is to analyses the germination rates and uniformity of seed germination of the *Berrya cordifolia*, which is a one of major plant species propagating in forest nurseries in Sri Lanka. Germination percentages and uniformity of seed germination will analyze under thirteen novel seed treatment methods. In order to optimize the seed pretreatment conditions for seed germination of *Berrya cordifolia*. Moreover, to discuss the best seed treatment options of *Berrya cordifolia*, which are suitable for Sri Lankan, forest nursery conditions. This study will be very important for the future propagations of *Berrya cordifolia* in Sri Lanka.

Materials and Methods

Studied species and the seed collection

As mentioned above I studied the seed germination of *Berrya cordifolia* (Trincomalee wood or Halmilla).

Seeds were collected from the mother tree located in the divisional forest office garden in Vavuniya located at the GPS point E: 169658.97 N: 39400.96, latitude WGS84 – 8.7550269 o Local – 8.754806253 o, Longitude WGS84 – 80.4979544 o Local – 80.49590392 o on 31st of December 2024. Specimen also collected from the mother tree and a herbarium specimen was prepared and deposited in



Figure 2. Mother tree of *Berrya cordifolia* located in the divisional forest office garden in Vavuniya.

Seeds were collected by hand picking method, without damaging to the mother tree. Only the fruits at dispersal maturity (brown-coloured dried fruits)

with no visual damages were collected. Fruits were brought to Forest nursery Vauniya and seeds were extracted by hand. The extracted seeds were stored at 0oC until initiation of experiments.



Figure 3. Seeds of *Berrya cordifolia* collected to use for the experiments.

Optimization of seed germination of *Berrya cordifolia* using seed pre-treatments.

The following seed pre-treatments were conducted to enhance the seed germination. Each treatment had five replicates of 50 seeds. A separate sample with five replicates of 50 non-treated seeds was used as the control. After the treatment seeds were incubated at room temperature and under the shade conditions in mother bed (1:1:1 (soil : sand : loam)) medium.

Pre-treatments used,

1. Treatment of bright sun light (between 12.00 p.m. to 1.00 p.m.).

This is a physical treatment primarily focus on light availability. The seeds of *B. cordifolia* were dried in bright sun light (between 12.00 p.m. to 1.00 p.m.) for one hour before sowing to improve the germination and seedling vigour.

2. Treatment of soaking *B. cordifolia* seeds along with a cotton bag in water.

This is a physical treatment primarily focus on water availability. The *B. cordifolia* seeds were soaked along with a cotton bag in 200 milliliters of water for 24 hours. The *B. cordifolia* seeds were dried in shade before introducing into mother bed.

3. Treatment in Pasgorasa/ Panchagavya (made of cow milk and it included a combination of items such as milk, ghee and wendaru) (07 ml per 200 ml of water).

The seeds were soaked in 200 ml of water for 12 hours. *B. cordifolia* seeds that are previously soaked in water for 12 hours were soaked in Pasgorasa (07 ml per 200 ml of water) 200 milliliters solution for another 06 hours. The *B. cordifolia* seeds were dried in shade before sowing in the mother bed.

4. Treatment in Soaking the seeds in cow dung extract.

This is a physical treatment primarily based on hydro priming and osmo priming (soaking the seeds on osmotic solution). The seeds were soaked in 200 ml of water for 12 hours. 100g of fresh cow dung was taken and diluted with one liter of water. Seeds of *B. cordifolia* were soaked in the cow dung extract (200ml) that are previously soaked in water for 12 hours. The seeds were soaked in this cow dung extract for 6 hours. Treated *B. cordifolia* seeds were dried in shade before sowing in the mother bed.

5. Treat the seeds with dried cow dung and cow dung powder.

This is a chemical treatment primarily focus on protect against pathogens. Seeds *B. cordifolia* were mixed with 100g of dried cow dung powder before introducing into mother bed.

6. Soak the seeds in 12% raw cow's milk (24 ml of raw cow's milk in 176 ml of water).

This is a physical treatment primarily based on hydro priming and osmo priming (soaking the seeds on osmotic solution). The seeds were soaked in 200 ml of water for 12 hours. *B. cordifolia* seeds that were previously soaked in water for 12 hours were again soaked in 200 ml of 12% raw cow's milk (24 ml of milk in 176 ml of water) for another 06 hours. The seeds were dried in shade and then introduce to the mother bed.

7. Soak seeds in warm water for 30 minutes before introduce in to mother bed.

This is a physical treatment primarily focus on temperature change/ heat shock. Soak the seeds of *B. cordifolia* tied in a cotton cloth in 200 ml of 60 °C (± 5) warm water for 30 minutes before introduce in to mother bed. This helps in the softening of the hard seed coat and will help to quicken the germination and result in fast growth and healthy plants.

8. Treat seeds with termite hill soil.

This is a chemical treatment primarily focus on protect against pathogens. Seeds of *B. cordifolia* were soaked in 200 ml of water for 12 hours. *B. cordifolia* seeds that are previously soaked in water for 12 hours were again soaked in termite hill soil solution (consist of equal amount of water and termite hill soil) 200 ml solution for another 06 hours. The *B. cordifolia* seeds were dried in shade before sowing in the mother bed. This will be important to get resistance for drought and grow luxuriantly.

9. Mix the seeds with salt water.

This is a physical treatment primarily based on hydro priming and osmo priming. Seeds of *B. cordifolia* were soaked in 200 ml of water for 12 hours. *B. cordifolia* seeds that are previously soaked in water for 12 hours were mixed with 200 ml of salt water (50 g of salt in 1 l of water). The *B. cordifolia* seeds were dried in shade before sowing in the mother bed.

10. Paste of ash and water and dry it in shade before sowing.

This is a chemical treatment primarily focus on protect against pathogens. Although this can consider as a physical treatment primarily based on hydro priming and osmo priming. All the seeds *B. cordifolia* were smeared with a paste of ash and water and dry it in shade before sowing. This treatment leads to control the seed borne diseases and enhance seed vigour and germination percentage.

11. Soak the seeds in a mixture of wheat flour, rice flour, turmeric and milk (50 g of each diluted in 1 l of fresh milk).

This is a physical treatment primarily based on hydro priming and osmo priming. The seeds were soaked in 200 ml of water for 12 hours. *B. cordifolia* seeds that were previously soaked in water for 12 hours were again soaked in a mixture of wheat flour, rice flour, turmeric and milk (50 g of each diluted in 1 l of fresh milk) for another 06 hours. The seeds were dried in shade and then introduce to the mother bed. This will enhance the germination percentage and growth rate.

12. Treat the seeds with buttermilk (125 g in 1 l of water).

This is a physical treatment primarily based on hydro priming and osmo priming. The seeds were soaked in 200 ml of water for 12 hours. *B. cordifolia* seeds that were previously soaked in water for 12 hours were again soaked in buttermilk (125 g in 1 l of water) for another 06 hours. The seeds were dried in shade and then introduce to the mother bed. This will also lead to prevent fungal diseases in crops.

13. Treatment in Soaking the seeds in rabbit fecal matter extract.

This is a physical treatment primarily based on hydro priming and osmo priming. The seeds were soaked in 200 ml of water for 12 hours. 100g of fresh rabbit fecal matter was taken and diluted with one liter of water. Seeds of *B. cordifolia* were soaked in the rabbit fecal matter extract (200 ml) that are previously soaked in water. The seeds were soaked in this rabbit fecal matter for 6 hours. The *B. cordifolia* seeds were dried in shade before sowing in the mother bed.

Germination parameters

Seeds were observed for germination in one day intervals to fourteen days. The following germination parameters were calculated using the following standard equations. Sprouting from the mother bed was the criterion for germination.

Germination percentage = Sprouted seeds/ Total seeds tested x 100

Germination speed (T50)

Germination speed according to the final population percentage was determined by the time to get to 50% of final population percentage (T50). Germination speed was determined by graphical method.

Germination speed (according to the final population percentage) = the time to get to 50% of final population percentage (T50)

Germination uniformity

Germination uniformity according to the final population percentage was calculated by the difference in germination times of 75% of final population percentage (T75) and 25% final population percentage (T25) as following equation.

Germination uniformity = $\frac{\text{Time consumed to germinate 75\% of final germinated population (T75)} - \text{Time consumed to germinate 25\% of final germinated population (T25)}}{\text{Time consumed to germinate 75\% of final germinated population (T75)}}$

(According to the final population percentage)

Data Analysis

Mean germination rates of each treatment and control test were calculated as follows.

Mean germination rate = sum of the germination rates/ number of replicates

The sample standard deviation was calculated as follows.

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2}$$

Here,

- s = Sample standard deviation symbol
- $\bar{x}$ or x = Arithmetic mean of the germination rates
- n = total number of germination rates

Mean germination speed of each treatment and control test were calculated as follows.

Mean germination speed = sum of the germination speeds/ number of replicates

The sample standard deviation was calculated as follows.

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2}$$

Here,

- s = Sample standard deviation symbol
- $\bar{x}$ or x^- = Arithmetic mean of the germination speeds
- n = total number of germination speeds

Mean germination uniformity of each treatment and control test were calculated as follows.

Mean germination uniformity = sum of the germination uniformities/ number of replicates

The sample standard deviation was calculated as follows.

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2}$$

Here,

- s = Sample standard deviation symbol
- $\bar{x}$ or x^- = Arithmetic mean of the germination uniformities
- n = total number of germination uniformities

Ranking of the seed treatments

The treatments used in this study were ranked according to seed germination percentage, seed germination speed and seed germination uniformity.

Results and discussion

Optimization of seed germination of *Berrya cordifolia* by novel seed pre-treatment techniques.

Observations were recorded 14 days from the day of introducing to the mother bed after the treatments. According to the observations on seed germination average germination percentage, average germination speed and average germination uniformity were calculated. In addition, morphological changes of the germinating seeds were recorded.



Figure 4. Germinated seed of *Berrya cordifolia*.

Average seed germination percentage

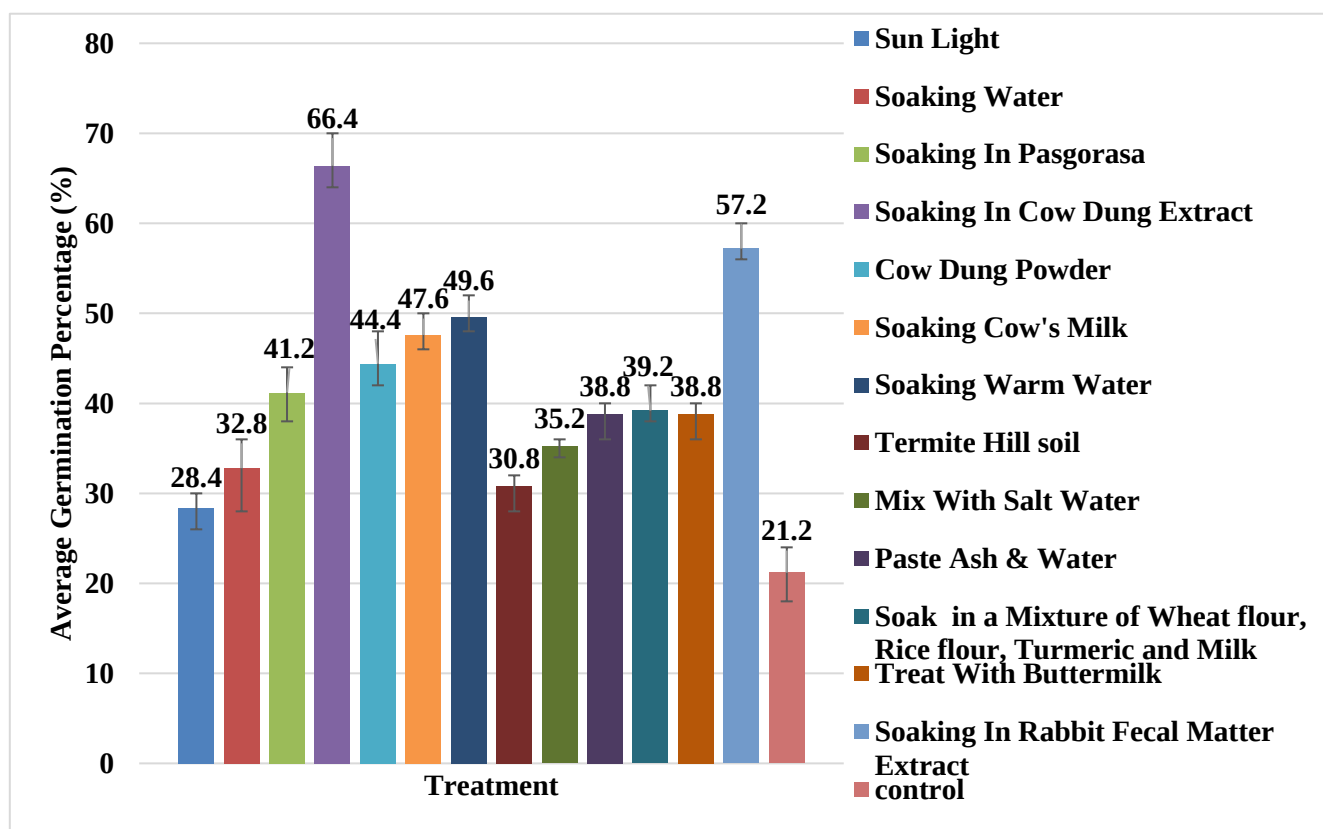


Figure 5. Germination percentages of *Berrya cordifolia* after 14 days from introducing to mother bed, under different seed treatment techniques.

In the present study, the maximum average seed germination percentage was found that treatment in soaking the seeds in cow dung extract for *Berrya cordifolia* seeds with 66.4 (± 2.61) % average germination percentage (Figure 05). Second highest average seed germination percentage was observed in treatment in soaking the seeds in rabbit fecal matter extract with 57.2 (± 1.78) % average germination percentage. Treatment of soaking in warm water is also having a considerable effect on seed germination percentage with 49.6 (± 1.67) % average germination percentage. Average seed germination percentages of soaking in Pasgorasa/Panchagavya, cow dung powder and soaking cow's milk treatments showed more than 40% of average germination percentages (41.2

(± 2.28) %, 44.4 (± 2.60) % and 47.6 (± 1.67) % respectively). However, the average germination speed of raw cow's milk and buttermilk treatments showed the considerable difference in average germination percentage (47.6 (± 1.67) % and 38.8 (± 1.78) % respectively). The average germination speed of paste ash and water and buttermilk treatments were equal (38.8 (± 1.78) %). Treatment of sun light showed the least effect on the seed germination percentage with 28.4 (± 1.67) % of average germination percentage. However, all the treatments tested here showed the improvement of seed germination percentage compared with the control experiment (Figure 06). The figures are statistically different from control.

Average seed germination speed

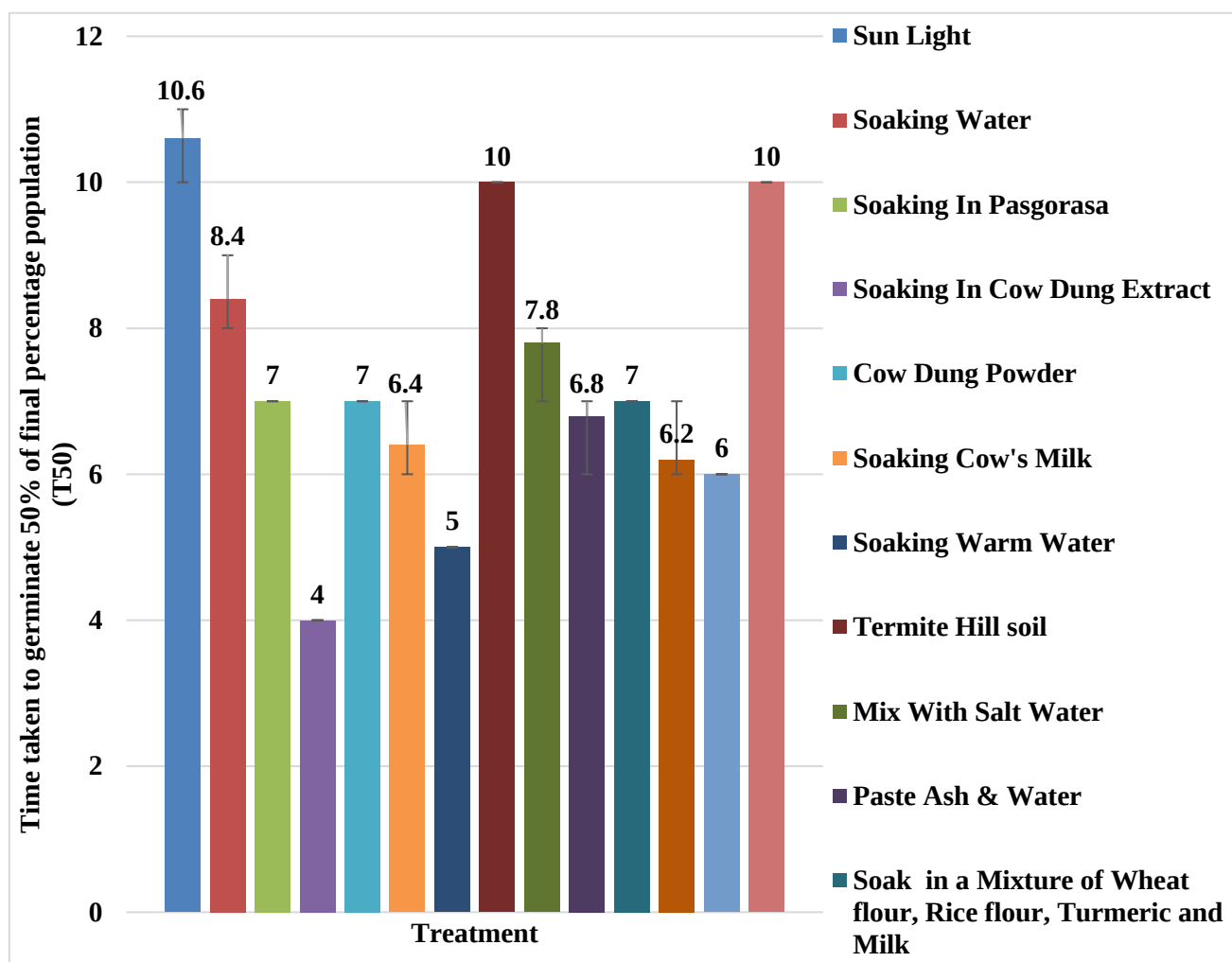


Figure 6. Time taken to germinate 50% of final percentage population (T50)/ germination speed of *Berrya cordifolia*, under different seed treatment techniques.

In the present study, the maximum average germination speed was 04 days from sowing (time taken to germinate 50% of final population) was found that treatment in soaking the seeds in cow dung extract for *Berrya cordifolia* seeds. Second highest average germination speed was 05 days from sowing (time taken to germinate 50% of final population) was observed in treatment in soaking the seeds in warm water. Treatment of soaking in rabbit fecal matter extract is also having a considerable effect on average germination speed with 6 days average germination speed. Average seed germination speeds of soaking cow's milk, paste ash and water treat with buttermilk and soaking in rabbit fecal matter extract took less than

7 days to germinate 50% of final population with average germination speeds 6.40 (± 0.54) days, 6.8 (± 0.44) days 6.2 (± 0.44) % and 6.0 days respectively. However, the average germination speed of soaking in cow dung extract and cow dung powder treatments showed the considerable difference in average germination speeds (04 days and 07 days respectively). Although the average germination speed of soaking in pasgorasa, cow dung powder and soak in a mixture of wheat flour, rice flour, turmeric and milk were equal (07 days). Treatment of sun light shows the negative effect on the seed germination speed with 10.60 (± 0.55) days of average germination speed. However, all other treatments expect the treatment of sunlight, tested

here showed the improvement of seed germination speed compared with the control experiment

(Figure 06). The figures are statistically different from control.

Average seed germination uniformity

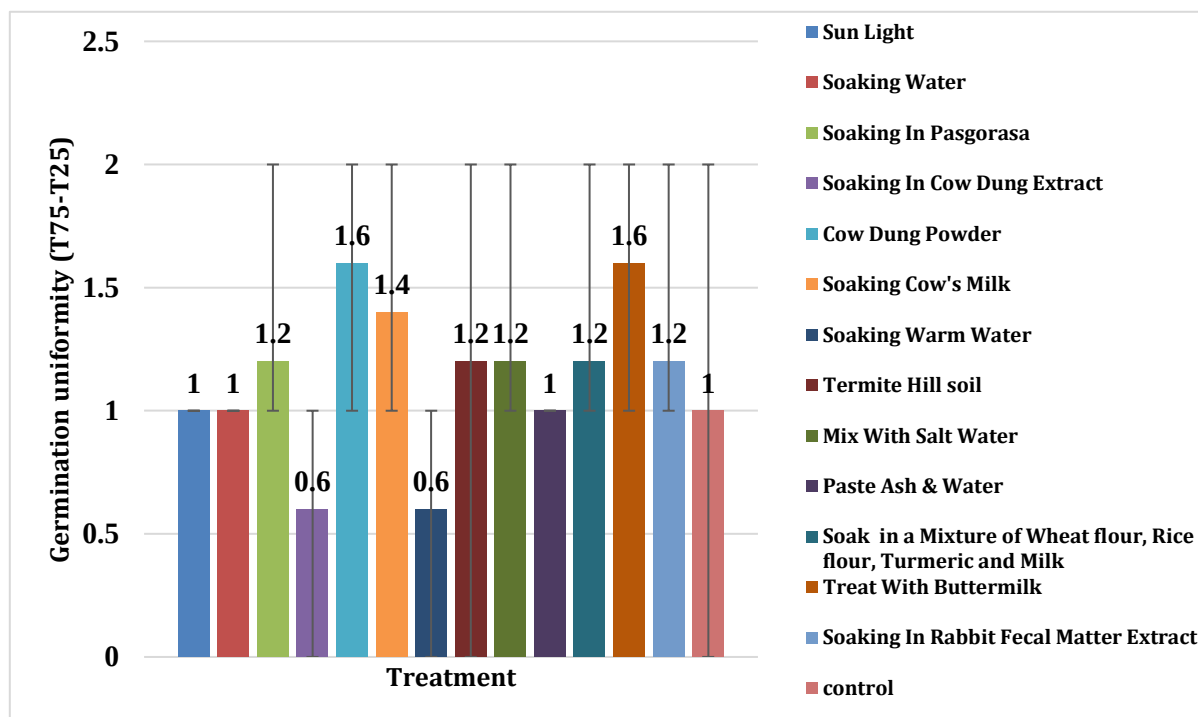


Figure 7. Germination uniformity (Time taken to germinate 75% of final percentage population (T75) - Time taken to germinate 25% of final percentage population (T25)) of *Berrya cordifolia*, under different seed treatment techniques.

In the present study, the maximum average germination uniformity 0.6 (± 0.54) days (difference between the time taken to germinate 75% of final population and time taken to germinate of 25% of final population) found in the treatment in soaking the seeds in cow dung extract and soaking in warm water for *Berrya cordifolia* seeds. Second highest average germination uniformity was 01 day (difference between the time taken to germinate 75% of final population and time taken to germinate of 25% of final population) were observed in treatments in treatment of sunlight, soaking the seeds in water, past ash and water and in control experiments. Soaking in pasgorasa; termite hill soil treatment; mix with salt-water treatment; soak in a mixture of wheat flour, rice flour, turmeric and milk and soaking in rabbit fecal matter extract show the equal seed germination uniformity (difference between the time taken to germinate 75% of

final population and time taken to germinate of 25% of final population) with 1.2 days. Soaking cow's milk treatment had low seed germination uniformity (difference between the time taken to germinate 75% of final population and time taken to germinate of 25% of final population was $1.4(\pm 0.55)$ days). However, least seed germination uniformity was observed in treatment with cow dung powder and treatment with buttermilk ($1.4(\pm 0.55)$ days). However, only soaking the seeds in cow dung extract and soaking in warm water tested here showed the improvement of seed germination uniformity compared with the control experiment (figure 08). The figures are statistically different from control.

Ranking of the seed treatments

Ranking based on seed germination percentage.

All the seed treatments used in this study were

ranked according to seed germination percentage as in following table.

Table 1. Ranking of treatments according to the average seed germination percentage.

Treatment	Average Germination Percentage (%)	Rank Number
Soaking in cow dung extract	66.4	1
Soaking in rabbit fecal matter extract	57.2	2
Soaking warm water	49.6	3
Soaking cow's milk	47.6	4
Cow dung powder	44.4	5
Soaking in pasgorasa	41.2	6
Soak in a mixture of wheat flour, rice flour, turmeric and milk	39.2	7
Paste ash & water	38.8	8
Treat with buttermilk	38.8	9
Mix with salt water	35.2	10
Soaking water	32.8	11
Termite hill soil	30.8	12
Sun light	28.4	13
control	21.2	14

According to the above results, all the treatments had positively effect on the seed germination. Among all, treatment of soaking in cow dung extract, soaking in rabbit fecal matter extract, soaking warm water, soaking cow's milk, cow dung powder treatment and soaking in pasgorasa showed high positive effect on seed germination percentage. All other treatments also showed a considerable

positive effect on seed germination percentage.

Ranking based on seed germination speed.

All the seed treatments used in this study were ranked according to seed germination speed as in following table.

Table 2. Ranking of treatments according to the average seed germination speed.

Treatment	Average Germination Speed (Days) (Time taken to germinate 50% of Final percentage of population)	Rank Number
Soaking In cow dung extract	4	1
Soaking warm water	5	2
Soaking In rabbit fecal matter extract	6	3
Treat with buttermilk	6.2	4
Soaking cow's milk	6.4	5
Paste ash & water	6.8	6

Soaking in pasgorasa	7	7
Cow dung powder	7	8
Soak in a mixture of wheat flour, rice flour, turmeric and milk	7	9
Mix with salt water	7.8	10
Soaking water	8.4	11
Termite hill soil	10	12
control	10	13
Sun light	10.6	14

According to the above results, except the treatment under the sunlight all the treatments had positively effect on the seed germination speed. Among all, treatment of soaking in cow dung extract, soaking in rabbit fecal matter extract, soaking warm water showed high positive effect on seed germination speed. All other treatments, except the treatment under the sunlight showed a considerable positive effect on seed germination speed. However, the

treatment under the sunlight showed negative effect on seed germination speed with high seed germination percentage compared with control experiment.

Ranking based on seed germination uniformity.

All the seed treatments used in this study were ranked according to seed germination uniformity as in following table.

Table 3. Ranking of treatments according to the average seed germination uniformity

Treatment	Average Germination Uniformity (Days) (Final percentage of population)	Rank Number
Soaking in cow dung extract	0.6	1
Soaking warm water	0.6	2
Sun light	1	3
Soaking water	1	4
Paste ash & water	1	5
control	1	6
Soaking in pasgorasa	1.2	7
Termite hill soil	1.2	8
Mix with salt water	1.2	9
Soak in a mixture of wheat flour, rice flour, turmeric and milk	1.2	10
Soaking in rabbit fecal matter Extract	1.2	11
Soaking cow's milk	1.4	12
Cow dung powder	1.6	13
Treat with buttermilk	1.6	14

According to the above results, only the treatment soaking in cow dung extract, soaking warm water, under the sunlight, soaking water and paste ash & water had positively effect on the seed germination uniformity. All other treatments showed negative effect on seed germination uniformity compared with control experiment.

Conclusion and Discussion

In this study, *Berrya cordifolia* seeds can be consider as photoblastic seeds. The heat is having considerable effect on seed germination. Treatment of soaking in pasgorasa can enhance the germination percentage about two times compared with the control experiment. However average germination speed was closer to the control experiment. Although average germination uniformity was, lower than the control experiment.

Treatment of soaking in cow dung extract was the best seed treatment tested in this study, in terms of average germination percentage, average germination speed and average germination uniformity. The germination percentage had increase more than three times compared with the control experiment. As well as average germination speed was more than twice of the control experiment. Although average germination uniformity was about two times than the control experiment.

Treatment in cow dung powder was one of the best seed treatment tested in this study, in terms of average germination percentage, average germination speed. However, average germination uniformity is lower than the control experiment. The germination percentage had increase more than two times compared with the control experiment. As well as average germination speed was below than twice of the control experiment. However, average germination uniformity was lower than the control experiment. Treatment of soaking in cow's milk was another best seed treatment tested in this study, in terms of average germination percentage, average germination speed. However, average germination uniformity is lower than the control experiment. The germination percentage had increase more than two times compared with the control experiment. As well as average germination speed was just below than twice of the control experiment. However, average germination

uniformity was lower than the control experiment.

Treatment of soaking warm water was the second-best seed treatment tested in this study, in terms of average germination percentage, average germination speed and average germination uniformity. The germination percentage had increase more than two times compared with the control experiment. As well as average germination speed was twice of the control experiment. However, average germination uniformity was also higher than the control experiment.

Treatment in termite hill soil was a treatment with high average germination percentage, equal average germination speed and low average germination uniformity compared with the control experiment.

Treatment of mix with salt water was a treatment with high average germination percentage, high average germination speed and low average germination uniformity compared with the control experiment.

Treatment of paste ash and water on seeds was a treatment with high average germination percentage, high average germination speed and equal average germination uniformity compared with the control experiment.

Treatment of soaking in a mixture of wheat flour, rice flour, turmeric and milk was a treatment with high average germination percentage, high average germination speed and low average germination uniformity compared with the control experiment.

Treatment of soaking in butter milk was a treatment with high average germination percentage, high average germination speed and low average germination uniformity compared with the control experiment.

Treatment of soaking in rabbit fecal matter extract was also can be consider as the second best seed treatment tested in this study, in terms of second highest average germination percentage and third highest average germination speed. However, average germination uniformity was low compared with the control experiment.

Each of the options for development of seed treatment techniques to exploit the economic and

temporal gain from the seed germination of *Berrya cordifolia* seeds have its own advantages and limitations. Whether they be seed germination percentage, speed and/or uniformity. Impotently through, the techniques are not mutually exclusive and could be pursued simultaneously.

However, significant variation was observed for seed germination percentages, speeds and uniformities between the seed treatment techniques used in this study. Of all the treatment techniques, the treatment of soaking in cow dung extract proved significantly superior to all other techniques used in this study for enhance seed germination percentage, speed and uniformity. Whilst financial benefits from this research could be realized immediately by using the results to guide the choice of seed treatment for forest nursery establishment for *Berrya cordifolia*, greater would be achieved by developing the seed germination capacity of above technique reported here, through one or more condition optimization options. The markedly superior performances of the treatment of soaking in cow dung extract over almost all other seed treatment techniques reported in this study should provide confidence that results of this study on germination capacity improvement of *Berrya cordifolia* is delivering significant gains in seed germination percentage, speed and uniformity to benefit of *Berrya cordifolia* growers.

According to the present study, second best seed treatments were soaking in rabbit fecal matter extract and soaking warm water. However, the germination speed and uniformity are low in the treatment of soaking in rabbit fecal matter extract. Therefore, it suggests the treatment of these three Series No. 5, Forest Research Centre, Sepilok, Sabah.

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best seed treatment techniques for *Berrya cordifolia* growers.

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