RESEARCH ARTICLE

Effect of priming with neem seed extract on seeds of four traditional rice varieties of Sri Lanka; Kaluheenati, Kurulurthuda, Madathawalu and Maa-wee

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Abstract: With the concern for environmentally friendly agriculture, demand for traditional rice varieties in Sri Lanka has been increased. Farmers claim that the seed germination in traditional rice varieties in Sri Lanka is remarkably low. Considering the reported benefits of neem in enhancing the germination, priming of rice seeds with distilled water and neem seed extracts (100, 50 and 25% strength) for 0, 24, 48, and 72 hours was used to enhance the performances in seed germination of four selected traditional rice varieties. Primed seeds were dried under ambient conditions until initial weight was achieved. Seed germination was tested at ambient laboratory conditions (~27 °C). Seed vigour was evaluated by measuring the root, and shoot lengths and seedling emergence in glasshouse conditions. Four replicates of 100 seeds were used in each treatment. Arcsine transformed data were analysed using one-way ANOVA. Germination percentages of un-primed seeds of Kaluheenati, Kuruluthuda, Maa-wee and Madathawalu were 62, 32, 24, and 20%, respectively, while seedling emergence of the same were 65, 10, 12 and 40%, respectively. Half strength neem priming (50%) for 24 hours and 24 hours pre-soaking significantly improved seed germination (83%) and seedling emergence (83%) of Kaluheenati, while quarter strength neem priming for 72 hours and 24 hours pre-soaking improved germination (64%) and seedling emergence (25%) of Kuruluthuda. Quarter strength neem priming for 48 hours, and 48 hours pre-soaking was effective in improving the germination (49%) and seedling emergence (30%) of Maa-wee, while priming with 100% neem extract for 72 hours and 48 hours pre-soaking improved the germination (55%) and seedling emergence (53%) of Madathawalu. Root and shoot lengths were higher in seedlings of neem primed seeds. Thus, priming with neem seed extracts can be recommended to

improve the performance in seed germination of studied rice varieties.

Keywords: Germination, neem priming, seed vigour.

INTRODUCTION

Rice is one of the most important staple foods in the world. Nevertheless, more than 90% of the world's rice is grown and consumed by Asian people (IRRI, 2020). As 60% of the world's population lives in Asia, rice could be considered as the world's most important food. Rice provides 50% of the dietary calorie supply for ~5 billion Asians (Muthayya *et al.*, 2014). Rice is cultivated in every continent except in Antarctica (Muthayya *et al.*, 2014). Moreover, rice is planted on about 148 million hectares annually, or on 11% of the world's cultivated land (Khush, 1997). Annually > 715 million tons of paddy rice is produced globally (FAO, 2013) showing its significance as a global crop. In Sri Lanka, rice was grown in 1,040,954 ha of land in the year 2018, while the production was 3,929,831 tons. However, only 1.22% of the rice grown lands has been devoted to produce traditional rice varieties of Sri Lanka (Department of Agriculture, 2019).

Traditional rice varieties that have been used by Sri Lankan farmers are with low yields. Thus, rice improvement programs in Sri Lanka have attempted to

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develop new rice varieties with higher yields. Most of these new varieties heavily dependent on agrochemicals (Rajapakse *et al.*, 2000). Nevertheless, the introduction of these new rice varieties caused the extinction of many traditional rice varieties from the field (Rajapakse *et al.*, 2000; Kennedy & Burlingame, 2003). Usage of extensive levels of agrochemicals is a significant problem as it has created many health and ecological problems. Most agrochemicals used in rice cultivation are not environmentally friendly (Bambaradeniya & Amarasinghe, 2003). These agrochemicals cause many human health problems (Weeraratne, 1983; Bandara *et al.*, 2008; 2010; Jayasumana *et al.*, 2014). Especially, with the emergence of chronic kidney disease with unknown aetiology, a new debate has been initiated in the country on the use of agrochemicals.

In the light of the current debate on health issues, organic farming, as well as traditional rice varieties were promoted as many of the traditional rice varieties are claimed to have health benefits (Dharmasena, 2010). Traditional rice varieties contain variety of chemicals with antioxidant properties. Thus, consumption of traditional rice varieties gives health benefits such as reducing the risk of non-infective diseases (Samaranayake *et al.*, 2017). Therefore currently, these traditional varieties have a good market value especially if they were produced organically (Wickramasinghe & Noda, 2008; Suriyagoda *et al.*, 2011).

However, low germinability and storability of seeds of the traditional rice varieties is a drawback in popularizing these varieties (*Personal communication with the organic farming community*). Thus, the current research was initiated on the request of the organic farming community to address the low germinability and storability issue of traditional rice varieties of Sri Lanka. In the current study, effect of priming on seed quality of four traditional rice varieties; Maa-wee, Kaluheenati, Kuruluthuda and Madthawalu were studied. No information was available on the improvement of seed quality of these rice varieties in scientific literature. However, few studies were conducted to reveal the medicinal (Samaranayake *et al.*, 2017) and physical (Ranawake *et al.*, 2013; Rebeira *et al.*, 2014) properties of these rice varieties.

Seed priming was used in this research to improve the seed quality of the selected species. Seed priming is a controlled hydration of seeds to initiate the molecular level process towards germination. Hydration is ceased prior to radicle emergence (Bradford & Bewley, 2002). Priming improves seed performance specially under stress conditions, inducing the rapid establishment of

vigorous rice seedlings (Tilahun *et al.*, 2013). Moreover, it improves the germination rate, growth of seedlings, and reduces the time to start germination (Drew & Dearman, 1993). Several seed priming methods are used to improve seed quality (Nawaz *et al.*, 2013); hydro-priming (use of water), osmopriming (use of solutions with low osmotic potentials), matrix priming (use of solid matrix; Harris *et al.*, 2002), nutrient priming (use of solutions with micronutrients; Johnson *et al.*, 2005) and hormonal priming (use of solutions with plant growth regulators; Ghobadi *et al.*, 2012).

Neem has been used as pesticide and as a treatment for many fungal and bacterial diseases in crops (Girish & Bhat, 2008). Isolated chemicals as well as neem seed extract have shown antibacterial (Coventry & Allan, 2001; Biswas *et al.*, 2002), antifungal (Achim & Schlosser, 1992; Coventry & Allan, 2001; Biswas *et al.*, 2002; Wang *et al.*, 2010) and anti-viral (Biswas *et al.*, 2002) properties. Further, neem seed extract has been reported to have significant antioxidant activity (Nahak & Sahu, 2011; Revathi & Thambidurai, 2019). Free radicle damage and pathogenic effects are the main factors reducing quality of seeds during storage (Copeland and McDonald, 2001). Thus, we hypothesized that antimicrobial and antioxidant effects of neem seed extract would improve the seed quality of traditional rice seeds. Furthermore, if neem seed extract is effective in improving seed quality it could be used as an organic treatment. Thus, the main objective of our study was to improve the seed quality of four selected traditional rice varieties in Sri Lanka with priming treatments using different concentrations of commercially available neem seed extract.

Four traditional rice varieties were used for the study. Three varieties (Madathawalu, Kaluhenati and Kuruluthuda) are cultivated in both Yala and Maha seasons whilst the other variety (Maa-Wee) is grown only in the Maha season (Ginigaddara, 2018). Kaluheenati and Madathawalu take 3 ½ months from sowing to harvest, while Kuruluthuda takes 4 months. In contrast, Maa-Wee require 5-6 months to get mature. Generally, Maa-Wee have a strong root system and healthy tillers. It is popular in Ratnapura, Kalutara, Galle, Matara and Gampaha districts (Personal communications with farmers). Madathawalu is suitable for muddy rice fields and cultivated in all districts. However, this variety is recommended by the Department of Agriculture for acidic soils. Kuruluthuda is better for flooded and high saline fields. Kaluheenati is suitable for puddled muddy lands and perform well in dry zone conditions (Ginigaddara, 2018).

METHODOLOGY

Seed materials

Seeds of the four studied rice varieties were obtained from HELA SAHAL Ayurvedic Seed Suppliers Pvt. Ltd., Kottawa, Sri Lanka on 4th of January 2017. Seeds were stored in polysac bags (710 × 480 mm² with 60 GSM thicknesses) until used for experiments. Seeds were from Maha cultivation season (cultivation season from September to March) 2016/2017 and were stored for only < 2 weeks by the supplier under ambient temperature conditions in gunny bags. Visually healthy seeds were selected for the field and laboratory experiments. Experiments were initiated within a week from the date of purchase. Field and laboratory experiments were conducted in the Department of Botany, University of Peradeniya. Experiments were initiated within 3 months from 6 January 2017 to April 2017.

Initial information for seed priming

Seed moisture content

Ten replicates of 10 seeds each from each variety were taken randomly and the seed samples were crushed separately using motor and pestle. Fresh weights of these crushed samples were measured using an analytical balance to the nearest 0.0001 g and were oven dried at 121°C for 3 h. Seed samples were reweighed, and moisture content was calculated in fresh mass basis (Fischer, 2007).

Imbibition of seeds

This experiment was conducted to evaluate the imbibition pattern of seeds to determine the suitable priming time. Four replicates of 20 seeds from each variety were weighed using a digital chemical balance to nearest 0.001 g. The seed samples were immersed in 30 ml of distilled water in beakers with aluminium foil lids and incubated at 25 °C. Seed samples were retrieved after 2, 4, 6, 8, 24, 48 and 72 h, surface blotted, reweighed and returned to the beakers. Imbibition curves were prepared.

Initial seed germination

Three samples containing three replicates of twenty seeds from each rice variety was taken randomly and incubated on tissue papers moistened with distilled water in 9 cm diameter Petri dish. One samples of each variety were

incubated at 25 °C in a temperature-controlled incubator [Model- MGC- 450BP, Company- Qualitron (Pvt) Ltd., Rajagiriya], at 35 °C in a temperature-controlled incubator [Model- MGC- 250P, Company- Qualitron (Pvt) Ltd., Rajagiriya] and the other at the ambient room temperature conditions. Samples were watered every day with distilled water and germinated seeds were counted for two weeks. Germination rate and the percentage were calculated.

Effect of seed priming on seed quality

Hydro-priming

Four replicates of 100 seeds from each variety were used for each priming treatment. Seed samples were weighed and immersed in 100 mL of distilled water in a 250 mL beaker. Seed samples were kept in distilled water for 0, 24, 48 and 72 h separately and were retrieved and reweighed. Then the seed samples were air dried until they came to their initial weight.

After the priming treatments, the vigour of the primed seeds was determined using the seedling growth test. Further, seed germination under laboratory conditions and seedling emergence under plant house conditions in rice field soils were studied after four pre-soaking treatments, i.e., 0 (no soaking), 24, 48 and 72 h soaking in distilled water as practiced by the traditional farmers.

Seed germination under the laboratory conditions

Hydro primed seed samples (from each priming treatment) and a non-primed sample from each species were incubated on manila papers moistened with distilled water. Manila papers with seeds were rolled and kept for germination under room temperature. Seeds were observed for germination after 7 d. The same experiment was conducted at 25 and 35 °C in temperature-controlled incubators.

Seed vigour test

Seed vigour of the primed seed samples were determined using the seedling growth criteria (Hampton & TeKrony, 1995). Twenty-five seedlings from each replicate of each sample mentioned above were selected randomly and were placed on a black coloured cloth and photographed. Root length and shoot length of the seedlings were measured using the images with the aid of Image J software.

Seedling emergence under the plant house conditions

Four replicates containing 100 primed (from each priming treatments separately) and non-primed seed samples of each species were pre-soaked in distilled water as explained above and incubated on moistened paddy field soil in separate $30 \times 45 \text{ cm}^2$ plastic trays at ambient plant house temperature conditions. Trays were watered every day for 7 d. Emerged seedlings were counted after 7 d. This experiment was conducted using only the best distilled water priming treatment and the best neem priming treatment as identified from seed germination and from seed vigour tests. Thus, the experiment was initiated two weeks after the initiation of the seed germination and seed vigour tests.

Seed priming with neem seed extract

Commercially available neem seed extract (Kohomba Saraya, 322/7, Negombo road, Kurunegala) was used, as neem seeds were not available at the time of initiation of the experiment. This commercially available neem seed extract is 100% natural as verified by the producers. According to the producer, neem seed extract has been prepared by crushing 5 kg of neem seeds and mixing with 10 L of water. This mixture has been kept overnight (12 h) and filtered. The filtrate was made up to 100 L by adding water.

Three samples containing four replicates with 100 seeds in each were selected randomly from each study rice variety and weighed with a digital chemical balance to the nearest 0.001 g. Seed samples were immersed in 100 mL of three different neem extract concentrations prepared (50 mL of neem solution mixed with 1 L of distilled water - full strength, 25 mL of neem solution mixed with 1 L of distilled water - half strength and 12.5 mL of neem solution mixed with 1 L of distilled water - quarter strength) from the commercially available neem seed solution.

Seed samples were allowed to imbibe neem solutions for 24, 48 and 72 h, after which seed samples were reweighed and air-dried until they came to their initial weight. Neem primed seeds samples were subjected to pre-soaking treatments as mentioned in hydro-priming. Then, seed germination at laboratory conditions, seed vigour, and seedling emergence under plant house conditions were studied as explained above. Different treatment combinations of priming and pre-soaking are depicted in figure 1.

Figure 1: Flow diagram depicting different hydro-priming and Neem-priming treatments and different pre-soaking treatments conducted in the research

Analysis of data

Arc-sine transformed germination percentage data, shoot and root length data and conductivity data were analysed using one-way and two-way ANOVA procedures. All the data were statistically analysed using Minitab 17 statistical software. All the graphs were drawn for non-transformed data using Sigma Plot 10.0 software.

RESULTS AND DISCUSSION

Initial information for seed priming

Seed moisture

Dry weights of studied rice varieties were between 1.72–2.33 g. Seed moisture contents of four traditional rice varieties were between 13–15% (Table 1).

Table 1: Average ($\pm$ SD: standard deviation) seed moisture content of the four rice varieties studied

Variety	Moisture content (%)
Maa-wee	13.7 $\pm$ 0.6
Kaluheenati	13.8 $\pm$ 0.8
Kuruluthuda	14.6 $\pm$ 1.7
Madathawalu	14.5 $\pm$ 1.3

Imbibition of seeds

Seeds of all the rice varieties increased in mass during the imbibition (Figure 2). Even after 72 hours of imbibition none of the seeds of different varieties reached the lag phase of seed germination.

Seed germination

Seeds with Kaluheenati germinated to ~ 80% at 25 °C and at ambient laboratory temperature conditions (Figure 3). Seeds of Madathawalu, Kuruluthuda and Maa-Wee germinated to ~60, 70 and 50%, respectively at these temperatures. However, at 35 °C, seeds of all the tested varieties germinated to < 20%.

Figure 2: Variations of the seed weight of rice varieties studied during imbibition with distilled water at ambient temperature conditions. Error bars $\pm$ SD

Figure 3: Cumulative germination of Madathawalu (A); Kuruluthuda (B); Maa-wee (C); and Kaluheenati (D) seeds at different temperature regimes (ambient laboratory temperature, and at 25 and 35 °C). Logistic 4-parameter curves were fitted to the germination percentage data to prepare the cumulative germination curves

Seeds of these four rice varieties, except Kaluheenati, had germination percentage <70% within 7 days at all the temperatures tested. The remaining seeds were soft and rotted within a short period. Low initial germination indicated that the initial quality of seeds of these four

varieties was low. Further, observed low germination percentages of seeds in studied varieties confirmed the claims of the traditional rice-growing farmers that seeds of traditional varieties had lower germinability. Even though the seeds were stored < 2 weeks by the supplier

and <1 week prior to the initiation of the experiments, seeds of Kuruluthuda, Maa-wee and Madathawalu had lost the germinability. Moreover, according to the results, seeds of all the varieties had low germination at 35 °C and thus we concluded that 35 °C is not favourable for seed germination of the traditional rice varieties that were tested. However, it is strange to see thermo-inhibition of rice germination at a temperature like 35 °C, as rice is a crop mainly cultivated in the tropical-subtropical regions of the world (Krishnan *et al.*, 2011). Several researchers have shown that the temperature requirement of rice seeds for germination depends on the rice variety (Tanida, 1996; Krishnan *et al.*, 2011).

Preliminary imbibition experiments showed that seeds of all the studied rice varieties would not have attained the lag phase (Bewley, 1997) of seed germination within 72 hours of the study period. Thus, we used seed priming treatments 24-, 48- and 72-hours duration in all the priming experiments conducted during the research.

Effect of priming on the quality of seeds of the study varieties

Seed germination at laboratory conditions

Kaluheenati: Non-primed Kaluheenati seeds germinated to 63% within 7 days, while most of the priming treatments

improved the germination percentage. However, the significantly highest germination percentage was observed in seeds primed for 24 hours with distilled water subjected to 24 hours pre-soaking treatment (86%) and 24 hours priming with a half strength of neem extract subjected to 24 hours pre-soaking treatment (85%, $F = 5.6$, $p < 0.01$, Table 2).

Kuruluthuda: Non-primed Kuruluthuda seeds reached only 32% cumulative germination within 7 days. However, significantly higher germination percentage was observed after 72 hours priming of seeds in distilled water followed with no pre-soaking treatment (55%), 72 hours priming of seed in half strength neem extract with no pre-soaking treatment (57%), 72 hours priming of seed in half strength neem extract followed by 48 hours soaking treatment (56%) and 72 hours priming of seed in quarter strength neem extract followed by 24 hours soaking treatment (64%, $F = 7.31$, $p < 0.01$; Table 2).

Maa-wee: Germination percentage of Maa-wee (unprimed 25% within 7 days) has significantly increased by many priming treatments. The highest germination was recorded after priming with quarter strength neem extract for 48 hours + 48 hours soaking (49%) (Table 2). Germination percentage after these priming treatments are significantly higher than the control ($F = 11.4$, $p < 0.01$).

Table 2: Average ($\pm$ SD) germination percentage of Kaluheenati, Kuruluthuda, Maa-wee and Madathawalu seeds after different priming treatments. Germination percentage in bold are significantly different from the germination percentage of the control

Treatment	Average ($\pm$ SD) of germination percentage			
	Kaluheenati	Kuruluthuda	Maa-wee	Madathawalu
no prime (no soak)	62.5 $\pm$ 1.4	32.4 $\pm$ 1.5	24.5 $\pm$ 2.9	20.0 $\pm$ 0.4
dw-24-0	72.3 $\pm$ 1.7	50.0 $\pm$ 0.8	22.2 $\pm$ 2.2	37.2 $\pm$ 1.3
dw-24-24	74.5 $\pm$ 1.2	50.2 $\pm$ 0.6	19.0 $\pm$ 1.4	33.5 $\pm$ 1.6
dw-24-48	79.8 $\pm$ 1.9	50.1 $\pm$ 3.2	24.5 $\pm$ 2.7	37.2 $\pm$ 2.5
dw-24-72	84.8 $\pm$ 1.6	50.3 $\pm$ 3.0	28.5 $\pm$ 1.5	34.7 $\pm$ 0.7
dw-48-0	75.8 $\pm$ 1.3	50.0 $\pm$ 1.8	29.5 $\pm$ 3.0	46.2 $\pm$ 2.7
dw-48-24	76.8 $\pm$ 0.9	38.0 $\pm$ 2.7	19.7 $\pm$ 2.2	45.7 $\pm$ 3.2
dw-48-48	79.3 $\pm$ 1.2	50.5 $\pm$ 1.4	25.2 $\pm$ 3.3	54.2 $\pm$ 2.0
dw-48-72	78.0 $\pm$ 0.7	51.0 $\pm$ 1.5	24.0 $\pm$ 3.3	45.7 $\pm$ 5.2
dw-72-0	77.8 $\pm$ 3.1	55.5 $\pm$ 1.1	27.5 $\pm$ 2.6	42.5 $\pm$ 2.1
dw-72-24	79.2 $\pm$ 1.0	50.0 $\pm$ 1.0	23.7 $\pm$ 1.6	43.7 $\pm$ 3.3
dw-72-48	78.3 $\pm$ 1.5	49.0 $\pm$ 1.2	32.5 $\pm$ 3.2	41.7 $\pm$ 2.4
dw-72-72	77.0 $\pm$ 1.3	50.0 $\pm$ 1.3	40.5 $\pm$ 2.9	47.3 $\pm$ 3.0

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Treatment	Average ($\pm$ SD) of germination percentage			
	Kaluheenati	Kuruluthuda	Maa-wee	Madathawalu
neem 1-24-24	81.8 $\pm$ 2.5	43.7 $\pm$ 1.1	30.7 $\pm$ 1.3	39.5 $\pm$ 1.7
neem 1-24-48	79.5 $\pm$ 0.9	46.7 $\pm$ 2.0	28.7 $\pm$ 2.5	41.5 $\pm$ 1.1
neem 1-24-72	81.0 $\pm$ 2.1	47.5 $\pm$ 1.1	30.0 $\pm$ 3.8	44.7 $\pm$ 2.0
neem 1-48-0	81.5 $\pm$ 1.9	49.5 $\pm$ 2.1	28.5 $\pm$ 2.1	42.0 $\pm$ 1.4
neem 1-48-24	83.8 $\pm$ 1.0	49.0 $\pm$ 2.1	34.0 $\pm$ 3.1	40.0 $\pm$ 2.2
neem 1-48-48	81.5 $\pm$ 2.5	51.0 $\pm$ 2.7	28.7 $\pm$ 2.2	38.5 $\pm$ 5.3
neem 1-48-72	78.0 $\pm$ 1.6	45.3 $\pm$ 2.6	33.2 $\pm$ 1.3	39.2 $\pm$ 1.4
neem 1-72-0	81.3 $\pm$ 1.4	43.0 $\pm$ 2.7	30.2 $\pm$ 3.8	38.2 $\pm$ 3.1
neem 1-72-24	81.3 $\pm$ 2.5	44.5 $\pm$ 1.3	31.7 $\pm$ 2.5	39.6 $\pm$ 3.8
neem 1-72-48	78.8 $\pm$ 1.7	46.5 $\pm$ 1.4	28.0 $\pm$ 2.0	55.0 $\pm$ 2.6
neem 1-72-72	76.5 $\pm$ 2.1	45.0 $\pm$ 2.0	37.7 $\pm$ 2.6	39.2 $\pm$ 3.1
neem 1/2-24-0	79.8 $\pm$ 2.3	48.5 $\pm$ 1.7	35.2 $\pm$ 2.5	45.5 $\pm$ 2.0
neem 1/2-24-24	85.5 $\pm$ 2.2	45.0 $\pm$ 2.4	40.7 $\pm$ 2.4	43.0 $\pm$ 3.1
neem 1/2-24-48	79.3 $\pm$ 1.1	45.7 $\pm$ 1.3	32.7 $\pm$ 1.6	39.9 $\pm$ 0.6
neem 1/2-24-72	78.9 $\pm$ 1.4	43.5 $\pm$ 2.6	38.2 $\pm$ 1.7	41.3 $\pm$ 1.2
neem 1/2-48-0	79.0 $\pm$ 2.5	52.7 $\pm$ 1.3	23.7 $\pm$ 1.4	47.7 $\pm$ 2.8
neem 1/2-48-24	83.3 $\pm$ 0.4	45.7 $\pm$ 2.7	20.5 $\pm$ 2.0	39.2 $\pm$ 2.2
neem 1/2-48-48	78.0 $\pm$ 2.4	51.0 $\pm$ 2.2	21.0 $\pm$ 1.5	42.5 $\pm$ 0.2
neem 1/2-48-72	74.4 $\pm$ 0.9	52.7 $\pm$ 1.8	28.0 $\pm$ 2.2	45.2 $\pm$ 2.2
neem 1/2-72-0	74.0 $\pm$ 0.5	56.5 $\pm$ 2.7	36.0 $\pm$ 0.9	37.7 $\pm$ 2.1
neem 1/2-72-24	77.5 $\pm$ 1.0	50.5 $\pm$ 3.4	34.0 $\pm$ 2.5	37.2 $\pm$ 1.6
neem 1/2-72-48	76.3 $\pm$ 1.9	55.7 $\pm$ 1.4	38.0 $\pm$ 1.6	39.0 $\pm$ 0.9
neem 1/2-72-72	76.8 $\pm$ 1.3	52.0 $\pm$ 0.9	40.5 $\pm$ 2.7	39.3 $\pm$ 2.2
neem 1/4-24-0	76.7 $\pm$ 2.2	50.2 $\pm$ 1.0	40.2 $\pm$ 1.8	39.5 $\pm$ 2.2
neem 1/4-24-24	80.7 $\pm$ 0.6	47.7 $\pm$ 1.4	41.2 $\pm$ 1.7	35.5 $\pm$ 3.2
neem 1/4-24-48	74.4 $\pm$ 2.4	46.0 $\pm$ 3.3	40.7 $\pm$ 2.6	37.5 $\pm$ 4.3
neem 1/4-24-72	75.0 $\pm$ 1.4	48.7 $\pm$ 4.0	37.5 $\pm$ 2.1	37.2 $\pm$ 3.3
neem 1/4-48-0	79.0 $\pm$ 2.7	48.2 $\pm$ 1.7	44.5 $\pm$ 1.8	38.5 $\pm$ 1.6
neem 1/4-48-24	78.5 $\pm$ 1.3	47.0 $\pm$ 2.1	44.7 $\pm$ 0.7	36.7 $\pm$ 2.2
neem 1/4-48-48	76.1 $\pm$ 1.7	49.0 $\pm$ 1.0	48.7 $\pm$ 1.8	38.7 $\pm$ 0.6
neem 1/4-48-72	74.7 $\pm$ 2.4	49.2 $\pm$ 1.3	40.0 $\pm$ 1.2	39.0 $\pm$ 1.5
neem 1/4-72-0	73.9 $\pm$ 0.8	45.0 $\pm$ 1.9	36.5 $\pm$ 1.5	39.9 $\pm$ 2.0
neem 1/4-72-24	75.5 $\pm$ 1.7	63.7 $\pm$ 3.9	37.5 $\pm$ 1.5	43.0 $\pm$ 2.7
neem 1/4-72-48	70.3 $\pm$ 1.7	57.0 $\pm$ 2.4	35.7 $\pm$ 1.8	40.7 $\pm$ 0.8
neem 1/4-72-72	72.5 $\pm$ 1.2	51.0 $\pm$ 1.4	41.7 $\pm$ 2.5	39.7 $\pm$ 2.2

*Treatment = priming agent-priming time (h)-pre-soaking time (h). dw = distilled water. neem 1, 1/2, 1/4 = neem extract full strength, half strength, quarter strength

Madathawalu: Cumulative germination of non-primed Madathawalu seeds (20% within 7 days) was improved by all the priming treatments (Table 2). However, significantly higher cumulative germination percentage

was observed in seeds after priming with full strength Neem extract for 72 hours + 48 hours soaking (55%) and priming with distilled water for 48 hours + 48 hours soaking (54%). ($F = 3.85$, $p < 0.01$).

Seed vigour of primed seeds

Kaluheenati: Root length (121.3 mm) of the seedlings developed from seeds primed in $\frac{1}{2}$ strength neem extract for 24 hours + 24 hours soaking were higher than those developed from non-primed and non-soaked seeds (86.8 mm), and those developed from seeds subjected to other priming and pre-soaking treatments ($F = 90.4$, $p < 0.001$, Figure 4). Shoot length followed a similar trend ($F = 17.5$, $p < 0.001$).

Figure 4: Mean of root (A) and shoot (B) lengths of Kaluheenati seedlings developed from seeds subjected to different priming and pre-soaking treatments. Different upper-case letters indicate significant differences between treatments. Error bars mean + SD. Only results of treatments that resulted in > 70 mm root and > 25 mm shoot length are shown in the figure.

Kuruluthuda: Seedlings with significantly higher root lengths ($F = 12.1$, $p < 0.001$) were produced by seeds primed with $\frac{1}{4}$ strength neem extract for 24 hours + 0 hours soaking, than those developed from non-primed

and non-soaked seeds (Figure 5). Same trend was observed in shoot length data ($F = 27.8$, $p < 0.001$).

Figure 5: Mean of root (A) and shoot (B) lengths of Kuruluthuda seedlings developed from seeds subjected to different priming and pre-soaking treatments. Different upper-case letters indicate significant differences between treatments. Error bars mean $\pm$ SD. Only results of treatments that resulted in > 60 mm root length and > 20 mm shoot length are shown in the figure.

Maa-wee: Root ($F = 17.7$, $p < 0.005$) and shoot lengths ($F = 33.4$, $p < 0.005$) of seedlings developed from seeds primed in quarter strength neem for 48 hours + 48 hours soaking were significantly higher than those developed from non-primed and non-soaked seeds and those developed from seeds subjected to other priming and pre-soaking treatments (Figure 6).

Madathawalu: Root lengths of seedlings developed from seeds primed with full strength neem extract for 72 hours + 48 hours soaking, and with full strength neem extract for 48 hours + 0 hours soaking were significantly higher than non-primed and non-soaked seeds (63 mm) and those developed from the seeds subjected to other priming and

soaking treatments ($F = 9.1$, $p < 0.005$, Figure 7). Shoot length (70.4 mm) of seedlings developed from seeds primed in full strength neem extract for 72 hours + 48

hours soaking was significantly higher ($F = 4.90$, $p < 0.005$) than those of non-primed and non-soaked seeds (32.4 mm).

Figure 6: Mean of root (A) and shoot (B) lengths of Maa-wee seedlings developed from seeds subjected to different priming and soaking treatments. Different upper-case letters indicate significant differences between treatments. Error bars mean $\pm$ SD. Only results of treatments that resulted in > 60 mm root and > 20 mm shoot length were shown in the figure.

Effect of priming on seedling emergence under plant house conditions

Kaluheenati

The seedling emergence percentage of non-primed seeds (65%) improved, significantly after priming with distilled water or neem extract (Figure 8A). However, the seedling emergence percentage of seeds primed in half strength neem extract for 72 hours followed by 24 hours soaking was significantly ($F = 28.9$, $p < 0.01$) higher than that of all the other treatments (82.5%).

Kuruluthuda

Percentage seedling emergence of non-primed Kuruluthuda seeds (10%) improved significantly ($F = 76.4$, $p < 0.01$) by distilled water (72 hours) or neem (quarter strength for 72 hours) priming (25%, Figure 8B).

Maa-wee

Seedling emergence of non-primed Ma-wee seeds was very low (12%, Figure 8C). However, when seeds were primed with quarter neem extract for 48 hours, seedling

emergence percentage (28%) was significantly improved ($F = 212.5, p < 0.01$).

Figure 7: Mean root (A) and shoot (B) lengths of Madathawalu seedlings developed from seeds subjected to different priming and soaking treatments. Different upper-case letters indicate significant differences between treatments. Error bars $\pm$ SD. Only results of treatments that resulted in > 70 mm root and > 30 mm shoot length are shown in the figure.

Madathawalu

Seedling emergence percentage of non-primed Madathawalu seeds was $\sim 40\%$. When seeds were primed with full strength Neem extract for 72 hours, seedling emergence percentage has significantly ($F = 29.9, p < 0.01$) increased to 54% (Figure 8D).

Non-primed Kaluheenati seeds had $\sim 60\%$ germinability. Among hydro-priming treatments, distilled water priming for 24 hours+ 72 hours pre-soaking treatment resulted the highest germinability, while among

neem priming treatments half strength neem extract priming for 24 hours+ 24 hours pre-soaking resulted the highest germinability. Neem priming treatment has improved the germination percentage of Kaluheenati seeds to about 85% . This percentage germinability was significantly higher than that of the hydro-primed seeds too. Further, the highest root and shoot length were observed after seeds were subjected to $\frac{1}{2}$ strength neem extract priming for 24 hours + 24-hour pre-soaking. Under glasshouse conditions, half strength neem extract priming for 24 hours+ 24 hours- pre-soaking treatment resulted the highest seedling emergence too. Therefore,

half strength neem extract priming for 24 hours + 24 hours pre-soaking could be recommended as the best treatment to improve seed quality of Kaluheenati.

Kuruluthuda had a very low initial germination ~35%. When seed germination percentage was considered, the best hydro-priming treatment was distilled water priming for 24 hours with no pre-soaking and the best neem priming treatments were quarter strength neem priming for 72 hours + 24 hours pre-soaking and half strength neem priming for 72 hours with no pre-soaking treatments. Neem priming treatments have improved the seed germinability significantly to ~65%. However, no significant difference was observed between distilled water priming for 24 hours with that of the two neem priming treatments. However, the highest root and shoot length were observed in seedlings of seeds primed with quarter strength neem extract for 72 hours + 24 hours pre-soaking treatment. However, quarter strength neem extract priming for 72 hours + 24 hours pre-soaking treatment as well as distilled water priming for 24 hours with no pre-soaking treatment has increased the seedling emergence over that of non-primed or primed with other treatments. Further, there was no significant difference

between seedling emergences of these two treatments. When all the germination parameters were considered, half strength neem extract priming for 72 hours + 24 hours pre-soaking was the best treatment for Kuruluthuda.

Maa-wee also had a low initial germinability (~25%). Distilled water priming for 72 hours+ 72 hours pre-soaking treatment was the best hydro-priming treatment that resulted the highest germinability. However, there were several neem priming treatments which improved the germinability of seeds of Maa-wee. Nevertheless, the germination percentage of seeds within seven days of this variety was always < 50% indicating the initial low quality of the seeds. Although neem priming has significantly increased the shoot and root lengths of seedlings, there were no significant differences between the shoot and root lengths of seedlings of neem primed seed with that of distilled water primed once. However, quarter strength neem extracts priming for 48 hours+ 48 hours pre-soaking treatment resulted in significantly the highest seedling emergence percentage. Therefore, it revealed that the best treatment to improve seed quality of Maa-wee is the quarter strength neem extract priming for 48 hours+ 48 hours pre-soaking treatments.

Figure 8: Seedling emergence of primed and non-prime seeds of (A) Kaluheenati, (B) Kuruluthuda, (C) Maa-wee and of (D) Madathawalu sown on rice field soil in ambient plant house conditions. Different uppercase letters indicate significant differences between treatments. Error bars mean $\pm$ SD.

Madathawalu had initial germination of ~20%. Distilled water priming for 48 hours + 48 hours pre-soaking treatment was the best hydro-priming treatment for Madathawalu to increase the seed germinability. Whereas full strength neem priming for 72 hours + 48 hours pre-soaking resulted the highest germinability among neem priming treatments. These treatments have significantly improved the germinability of Madathawalu seeds to about 55% but with no significant differences of germinability between the best hydro-priming and neem priming treatments. However, seed vigour (as determined with the shoot and root length) of seeds with full strength neem priming for 72 hours + 48 hours pre-soaking was significantly higher than that of the seeds hydro-primed for 48 hours + pre-soaking for 48 hours. Thus, full strength neem seed extract priming for 72 hours + pre-soaking for 48 hours could be considered as the best treatment for Madathawalu. However, there is no significant difference between seedling emergences of seed primed with full strength neem extract for 72 hours + 48 hours pre-soaking and that of 72 hours hydro-priming + 72 hours pre-soaking.

The results of the priming experiments revealed that neem priming has a higher ability than water priming to improve seed quality of Kaluheenati, Kuruluthuda, Maa-wee and Madathawalu. There could be basically three reasons for reduced seed quality: imbibition stress during germination, antioxidant stress and pathogenic activity. During imbibition, if water enters to seeds without a control, it could cause reactive oxygen species (ROS) accumulation ((Liu *et al.*, 2007, Paparella *et al.*, 2015). ROS could damage cellular components (Paparella *et al.*, 2015). The reasons for the increment of seed quality (seed germination and seed vigour) with distilled water may be that it reduces imbibition stress. Neem extract has a lower water potential than distilled water. Therefore, it controls (reduces) water uptake. It could limit ROS formation and thereby reduce cellular damages and oxidative injuries. Further, neem seed extracts show antioxidant properties (Nahak & Sahu, 2011; Revathi & Thambidurai, 2019) and may reduce the effect of ROS during germination. Moreover, the anti-microbial activity of neem seed extract (Biswas *et al.*, 2002) may reduce the pathogenic activities in the seed during germination. These may be the reasons for seed quality improvement shown after neem priming in our experiments. However, a detailed study has to be conducted to determine the mode of action of the neem seed extract during seed priming.

No studies on seed germination/vigour tests have been reported on the studied four rice varieties. However, several studies have been conducted to determine the

effects of hydro-priming, dehydration priming and osmo-priming (by NaCl and KH_2PO_4) on germination of rice seeds. Galappaththi *et al.* (2020) have reported that hydro-priming for 72 hours significantly improve the seed germination, vigour and emergence of two traditional rice varieties; Suwadal and Batapola-el. Illangakoon *et al.* (2016) have showed that seed hydro-priming has improved the survival of both traditional and newly developed varieties under anaerobic conditions. They further reported that, hydro-primed seeds showed increased synthesis of soluble sugars, starch degradation and α amylase activity compared to non-primed seeds under anaerobic conditions. They suggested that these enhanced activities are the modes by which hydro-priming enhanced seed quality. Islam *et al.* (2012) have reported that CaCl_2 was the best osmo-priming agent. Priming using CaCl_2 enhanced all germination parameters of BRRI dhan41 rice variety in Bangladesh. Seeds of BRRI dhan41, when treated with NaCl, showed the highest germination percentage, germination energy, germination speed. However, vigour index was found to be highest in BRRI dhan40 when it was treated with KCl. BRRI dhan41 produced the largest root when seeds were treated with NaCl. BRRI dhan40 and BINA dhan7 produced the highest shoot length at the controlled and CaCl_2 treated seed, respectively. Esmeili and Heidarzade, (2012) have found that the osmo-priming mainly with PEG could improve seed germination and seedling development parameters of many rice genotypes.

Heretofore, neem priming has not been used to improve seed quality of any species. Our experiments showed the high potential of neem priming in the improvement of seed quality of traditional rice varieties. This finding is especially important as neem can be used as an organic agrochemical in organic farming. Thus, our experiments provide a solution to a basic problem of low seed quality faced by organic farmers who produce organic traditional rice.

According to the results obtained from our experiments, it revealed that hydro priming and neem priming are promising methods to enhance seed quality of all four traditional rice varieties studied. Priming treatments increase seed germinability, seed vigour and seedling emergence. Neem priming gives a more significant improvement of all these seed parameters thus, neem priming is more promising than hydropriming in improving seed quality of traditional rice varieties. However, it is necessary to determine how the antioxidant activity and other properties of neem affect the seed quality of rice.

CONCLUSIONS

Neem-priming treatment improved the seed quality (germination and vigour) of Kaluheenati, Kuruluthuda, Maa-wee and Madathawalu rice varieties. For Kaluheenati, the best results were obtained by half strength neem extract priming for 24 hours +24 hours water pre-soaking. For Kuruluthuda quarter strength neem extract priming for 72 hours + 24 hours water pre-soaking, for Maa-wee quarter strength neem extract priming for 48 hours +48 hours water pre-soaking and for Madathawalu full strength neem extract priming for 72 hours + 48 hours water pre-soaking, gave the best results.

REFERENCES

- Achimu P. & Schlosser E. (1992). Effect of neem extracts against *Plasmopara viticola*, *Practise Oriented Results on Use and Production of Neem Ingredients* (ed. H. Kleeberg), pp. 99–109. Druck & Graphic, Giessen, Germany.
- Alabi O. & Olorunju P.E. (2004). Evaluation of neem seed extract, black soap and cow dung for the control of groundnut leaf spot at Samaru, Nigeria. *Archives of Phytopathology and Plant Protection* **37**: 123–127. DOI: <https://doi.org/10.1080/0323540042000203921>
- Bambaradeniya C.N.B. & Amerasinghe F.P. (2003). Biodiversity associated with the rice field agroecosystem in Asian countries: A brief review. *Working Paper* 63. International Water Management Institute, Colombo.
- Bandara J.M.R.S., Senevirathna D.M.A.N., Dassanayake D.M.R.S.B., Herath V., Bandara J.M.R.P., Abeyssekara T. & Rajapaksha K.H. (2008). Chronic renal failure among farm families in cascade irrigation systems in Sri Lanka associated with elevated dietary cadmium levels in rice and freshwater fish (Tilapia). *Environmental Geochemistry and Health* **30**(5): 465–478. DOI: <https://doi.org/10.1007/s10653-007-9129-6>
- Bandara J.M.R.S., Wijewardena H.V.P., Liyanage J., Upul M.A. & Bandara J.M.U.A. (2010). Chronic renal failure in Sri Lanka caused by elevated dietary cadmium: Trojan horse of the green revolution. *Toxicology Letters* **198**(1): 33–39. DOI: <https://doi.org/10.1016/j.toxlet.2010.04.016>
- Bewley J.D. (1997). Seed germination and dormancy. *The Plant Cell* **9**(7): 1055–1066.
- Biswas K., Chattopadhyay I. & Banerjee R.K. (2002) Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science* **82**: 1336–1345.
- Bradford K.I. & Bewley J.D. (2002). Seeds: biology, technology and role in agriculture. In: *Plants, Genes and Crop Biotechnology* (eds. M.J. Chrispeels & D.E. Sadava), pp. 210–239, 2nd edition, Jones & Barlett. Boston, MA, USA
- Copeland L.O. & McDonald M. (2001). *Principals of Seed Science and Technology*. Springer Nature Publications, Switzerland.
- Coventry E. & Allan E.J. (2001). Microbiological and chemical analysis of neem (*Azadirachta indica*) extracts: new data on antimicrobial activity. *Phytoparasitica* **29**: 441–449. DOI: <https://doi.org/10.1007/BF02981863>
- Department of Agriculture (2019). *Agricultural Statistics*, volume 16. Socio Economic and Planning Centre, Department of Agriculture, Peradeniya.
- Dharmasena P.B. (2010) Traditional rice farming in Sri Lanka, *Economic Review* **36**: 48–53.
- Drew R. L. K. & Dearman J. (1993). Effect of osmotic priming on germination characteristics of celeriac (*Apiumgraveolens* L. var. *rapaceum*). *Seed Science and Technology* **21**(2): 411–415.
- Esmeili M. & Heidarzade A. (2012). Osmopriming enhance the seeds emergence and seedling parameters of two rice cultivars (*Oryza sativa*) under tough condition. *International Journal of Agricultural Crop Science* **4**(5): 247–250.
- Food and Agricultural Organisation (FAO). 2013. FAOSTAT Database. Food and Agricultural Organization, Rome, Italy.
- Fischer K. (2007). Methods of moisture testing. In: *ISTA Handbook on Moisture Determination* (eds. H. Nijenstein, J. Nydam & R. Don). International Seed Testing Association, Switzerland.
- Galappaththi M.O., Jayasuriya K.M.G.G. & Gama-Arachchige N.S. (2020). Storage and hydro-priming treatments to improve the seed quality of two traditional rice varieties; Batapola-el and Suwendal, from Sri Lanka. *Oryza-An international Journal on Rice* **57**(1): 25–35. DOI <https://doi.org/10.35709/ory.2020.57.1.3>
- Ghobadi M., Abnavi M.S., Honarmand S.J., Ghobadi M.E. & Mohammadi G.R. (2012). Effect of hormonal priming (GA3) and osmopriming on behavior of seed germination in wheat (*Triticum aestivum* L.). *Journal of Agricultural Science* **4**(9): 244. DOI: <https://doi.org/10.5539/jas.v4n9p244>
- Ginigaddara G.A.S. (2018). *Traditional Rice Varieties in Sri Lanka*. Rajarata University of Sri Lanka, Mihinthale, Sri Lanka. Available at http://www.rjt.ac.lk/agri/agri_onlinematerials/agricultural_systems/Traditional%20rice%20varieties%20GAS%20Ginigaddara.pdf
- Girish K. & Bhat S.S. (2008). Neem, a green treasure. *Electronic Journal of Biology* **4**: 102–111.
- Hampton J.G. & TeKrony D.M. (1995). *Handbook of Vigour Testing Methods*, 3rd edition. International Seed Testing Association, Switzerland.
- Harris D., Tripathi R.S. & Joshi A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. In: *Direct seeding: Research Strategies and Opportunities*, pp. 231–240. International Research Institute, Manila, Philippines.
- Illangakoon T.K., Ella E.S., Ismail A.M., Marambe B., Keerthisena R.S.K., Bentota A.P. & Kulatunge S. (2016). Impact of variety and seed priming on anaerobic germination-tolerance of rice (*Oryza sativa* L.) varieties in Sri Lanka. *Tropical Agricultural Research* **28**: 26–37. DOI: <https://doi.org/10.4038/tar.v28i1.8181>

- International Rice Research Institute (IRRI) (2021). *World Rice Statistics 2020*. International Rice Research Institute, Los Banos, Philippines. Available at http://irri.org/index.php?option=com_k2&view=item&id=9081&Itemid=100481&lang=en. Accessed 09 April 2020.
- Islam R., Mukherjee A. & Hossain M. (2012). Effect of osmopriming on rice seed germination and seedling growth. *Journal of the Bangladesh Agricultural University* **10**(1): 15–20.
DOI: <https://doi.org/10.3329/jbau.v10i1.12013>
- Jayasumana C., Gunatilaka S. & Senanayake P. (2014). Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemics of chronic kidney disease of unknown etiology in Sri Lanka. *International Journal of Environmental Research and Public Health* **11**(2): 2125–2147.
DOI: <https://doi.org/10.3390/ijerph110202125>
- Johnson S.E., Lauren J.G., Welch R.M. & Duxbury J.M. (2005). A comparison of the effects of micronutrient seed priming and soil fertilization on the mineral nutrition of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) in Nepal. *Experimental Agriculture* **41**: 427–448.
DOI: <https://doi.org/10.1017/S0014479705002851>
- Kennedy G. & Burlingame B. (2003). Analysis of food composition data on rice from a plant genetic resource perspective. *Food Chemistry* **80**(4): 589–596.
DOI: [https://doi.org/10.1016/S0308-8146\(02\)00507-1](https://doi.org/10.1016/S0308-8146(02)00507-1)
- Khush G.S. (1997). Origin, dispersal, cultivation and variation of rice. *Oryza: From Molecule to Plant* **35**(1): 25–34.
DOI: <https://doi.org/10.1023/A:1005810616885>
- Krishnan P., Ramakrishnan B., Reddy K.R. & Reddy V.R. (2011). High temperature effects on rice growth, yield, and grain quality. In: *Advances in Agronomy* (ed. D.L. Sparks), pp. 87–206, volume 111, Academic Press, Burlington, Canada.
DOI: <https://doi.org/10.1016/B978-0-12-387689-8.00004-7>
- Liu X., Xing D., Li L. & Zhang L. (2007). Rapid determination of seed vigor based on the level of superoxide generation during early imbibition. *Photochemical & Photobiological Sciences* **6**(7): 767–774.
DOI: <https://doi.org/10.1039/b704337f>
- Muthayya S., Sugimoto J.D., Montgomery S. & Maberly F. (2014). An overview of global rice production, supply, trade and consumption. *Annals of the New York Academy of Sciences* **1324**: 7–14.
DOI: <https://doi.org/10.1111/nyas.12540>
- Nahak G. & Sahu R. (2011). Evaluation of antioxidant activity of flower and seed oil of *Azadirachta indica* A. juss. *Journal of Applied and Natural Science* **3**: 78–81.
DOI: <https://doi.org/10.31018/jans.v3i1.158>
- Nawaz J., Hussain M., Jabbar A., Nadeem G.A., Sajid M., Subtain M. & Shabbir I. (2013). Seed priming techniques. *International Journal of Agriculture Crop Science* **6**(20): 1373–1381.
- Paparella S., Araújo S.S., Rossi G., Wijayasinghe M., Carbonera D. & Balestrazzi A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Reports* **34**(8): 1281–1293.
DOI: <https://doi.org/10.1007/s00299-015-1784-y>
- Rajapakse R.M.T., Sandanayake C.A. & Pathinayake B.D. (2000) Footprints in rice variety improvement and its impact on rice production in Sri Lanka. *Annual Symposium of the Department of Agriculture*, Department of Agriculture, Sri Lanka **2**: 423–433.
- Ranawake A.L., Amarasinghe U.G.S. & Dahanayake N. (2013). Agronomic characters of some traditional rice (*Oryza sativa* L.) cultivars in Sri Lanka. *Journal of the University of Ruhuna* **1**(1): 3–9.
DOI: <https://doi.org/10.4038/jur.v1i1.6150>
- Rebeira S.P., Wickramasinghe H.A.M., Samarasinghe W.L.G. & Prashantha B.D.R. (2014). Diversity of grain quality characteristics of traditional rice (*Oryza sativa* L.) varieties in Sri Lanka. *Tropical Agricultural Research* **25**(4): 570–578.
DOI: <https://doi.org/10.4038/tar.v25i4.8062>
- Revathi T. & Thambidurai S. (2019). Cytotoxic, antioxidant and antibacterial activities of copper oxide incorporated chitosan-neem seed biocomposites. *International Journal of Biological Macromolecules* **139**: 867–878.
DOI: <https://doi.org/10.1016/j.ijbiomac.2019.07.214>
- Samaranayake M.D.W., Yathursan S., Abeysekera W.K.S.M. & Herath H.M.T. (2017). Nutritional and antioxidant properties of selected traditional rice (*Oryza sativa* L.) varieties of Sri Lanka. *Sri Lankan Journal of Biology* **2**(2): 25–35.
DOI: <https://doi.org/10.4038/slj.b.v2i2.10>
- Schmutterer H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology* **35**(1): 271–297.
DOI: <https://doi.org/10.1146/annurev.en.35.010190.001415>
- Suriyagoda L.D.B., Thilakarathne R.M.M.S., Nissanka S.P. & Samita S. (2011). Morphological variation in selected rice (*Oryza sativa* L.) germplasm of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka* **39**(2): 129–137.
DOI: <https://doi.org/10.4038/jnsfsr.v39i2.3173>
- Tanida M. (1996). Catalase activity of rice seed embryo and its relation to germination rate at a low temperature. *Breeding Science* **46**(1): 23–27.
DOI: <https://doi.org/10.1270/jsbbs1951.46.23>
- Tilahun-Tadesse F., Nigussie-Dechassa R., Bayu W. & Gebeyehu S. (2013). Effect of hydro-priming and pre-germinating rice seed on the yield and terminal moisture stress mitigation of rain-fed lowland rice. *Agriculture, Forestry and Fisheries* **2**(2): 89–97.
DOI: <https://doi.org/10.11648/j.aff.20130202.15>
- Wang J., Li J., Cao J. & Jiang W. (2010). Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. *African Journal of Microbiology Research* **4**: 1100–1104.
- Weeraratne C.S. (1983). Pesticides, an overview with particular reference to Sri Lanka. *Economic Review* **8**: 4–12.
- Wickramasinghe H.A.M. & Noda T. (2008). Physicochemical properties of starches from Sri Lankan rice varieties. *Food Science and Technology Research* **14**(1)
DOI: <https://doi.org/10.3136/fstr.14.49>