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# Augmentation of Seedling Vigour using Biostimulants for Cowpea var VBN 4

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## ABSTRACT

As part of this research, an investigation was conducted to assess the impact of utilizing natural biostimulants on the biochemical and seedling characteristics of cowpea seeds variety VBN 4. Specifically, the study aimed to determine the most capable biostimulant in promoting enzyme activity and seedling vigor when applied at a 5% concentration for a period of eight hours. A total of eight different biostimulants were utilized in treating the seeds, and their outcomes were compared to those of untreated control seeds. The results revealed that humic acid at a 5% concentration and an eight-hour treatment period had the highest values for both enzyme activity and seedling vigor. Following closely behind were *Pseudomonas fluorescens* and brown kelp extract, showcasing their promising qualities as biostimulants. Overall, this study highlights the significant benefits that biostimulants offer to farmers as a means to increase seed viability and vigor. Based on these findings, it can be concluded that incorporating natural biostimulants into agricultural practices is a valuable strategy for improving crop yield and overall plant health.

**Keywords** Biostimulants, Cowpea, Humic acid, Seedling vigor, Enzyme activity.

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## INTRODUCTION

The cowpea plant, or *Vigna unguiculata* L., Walp, is a legume that can withstand various challenges in the agricultural environment. It is frequently grown in areas with high temperatures and limited water supplies (Sindhu *et al.* 2019). Grains are a versatile food source that contains high-energy compounds that improve human health and fight malnutrition. Their physiological quality determines the crop's establishment as well as its productivity (Bagateli *et al.* 2019). Superior seeds are better able to produce consistent seedlings that will eventually develop into individuals with abundant productive potential and economical use of the environment's resources.

A number of anatomical and compositional factors including seed coat thickness and structure, seed size, hilum size, and protein content, become increasingly significant in the complex process of cowpea water uptake. The degree to which the testa interlocks with the cotyledons can limit their ability to absorb water (Sefa-Dedeh and Stanley 1979). For fast germination, we must concentrate on treating seeds with either organic or inorganic liquids. Thus, the focus of our research was biostimulants.

Natural plant biostimulants (PBs) are a promising and environmentally friendly innovation that improve a variety of abiotic stressor tolerance while also enhancing flowering, fruit set, nutrient use efficiency, plant growth and productivity (Colla and Rouphael 2015). These biologically derived products contain growth regulators, essential nutrients, and protective compounds that promote plant growth and productivity. But these bio-stimulators' stimulatory effect depends entirely on the plant's source, dosage, and stage at which the extract was applied. The definition of PBs has been rigorously debated over the last decade and recently under the new Regulation (EU) 2019/ 1009, which led to the following: "A plant biostimulant shall be an EU fertilizing product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizosphere" (EU 2019).

Given this context, the current study's goal was to improve the physiological and biochemical characteristics of cowpea seeds using various biostimulants.

## MATERIALS AND METHODS

Genetically pure and fresh cowpea var VBN 4 seeds were collected from National Pulses Research Center, Vamban, Pudukkottai, Tamilnadu. Totally eight biostimulants solutions were gathered for seed treatment. Powder samples of Kelp extract (Brown and Red) were collected from the Faculty of Marine Sciences at Annamalai University Parangipettai. Chitosan bought from BFCLAB ®. The remaining materials (humic acid, moringa and neem leaf) were gathered from the agriculture faculty, while Rhizobium and *Pseudomonas fluorescens* were acquired from the Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University.

Initially, we collect the leaf samples (moringa, neem and kelps) then it's washed and shade dried for 2 days. After that leaf samples were powered using a grind jar in the Molecular laboratory in the Department of Genetics and Plant Breeding. The powdered samples were mixed with distilled water using a magnetic stirrer with heat. After 30 minutes of cooling, we were using the cooling centrifuge at 10,000 rpm for 10 minutes to collect the pure extract. The remaining was already in liquid form, so we focused on soaking duration and concentration (5%). The seeds were soaked in solutions for 8 hours with a 1:3 ratio (v/w).

Treatment details:

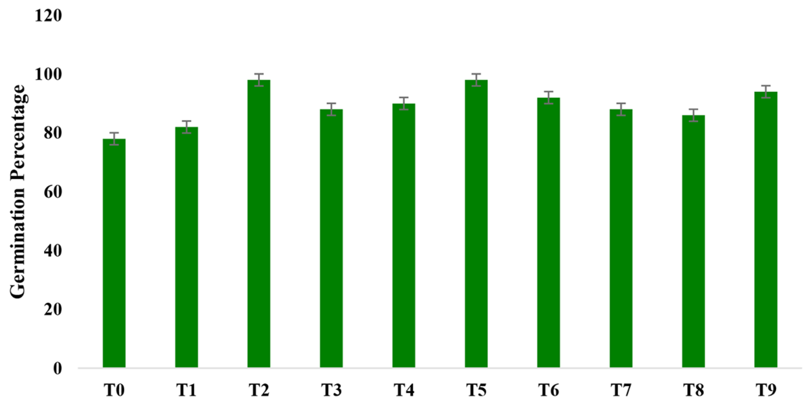
$T_0$ – Dry
$T_1$– Water soaked
$T_2$ – Humic acid 5%
$T_3$ – Chitosan 5%
$T_4$ – Kelp extract (Red) 5%
$T_5$ – Kelp extract (Brown) 5%
$T_6$ – Moringa leaf extract 5%
$T_7$– Neem leaf extract 5%
$T_8$– Rhizobium 5%
$T_9$– *Pseudomonas fluorescens* 5%

After the priming treatments the seeds were shade dried to the original moisture content of 9% and germination test was conducted with four replicates of 100 seeds in paper towels (ISTA 1999). The test conditions were 25 ± 2ºC temperature, 95±5% Relative Humidity and illumination with fluorescent light (750-1250 lux). A final count of normal seedlings was recorded on the 8th day.

Observations on germination percentage, seedling length and vigor index were recorded. Biochemical parameters viz., dehydrogenase activity (Kittock and Law 1968) was expressed as OD value @ 480 nm and α-amylase activity expressed as mg maltose $min^{-1}$ (Paul *et al.* 1970) respectively. All analyses were made in duplicate. AGRES software was used to statistically analyze the data. When needed, percentage data were converted to arcsine values.

## RESULTS AND DISCUSSION

The best treatments were determined by the pilot

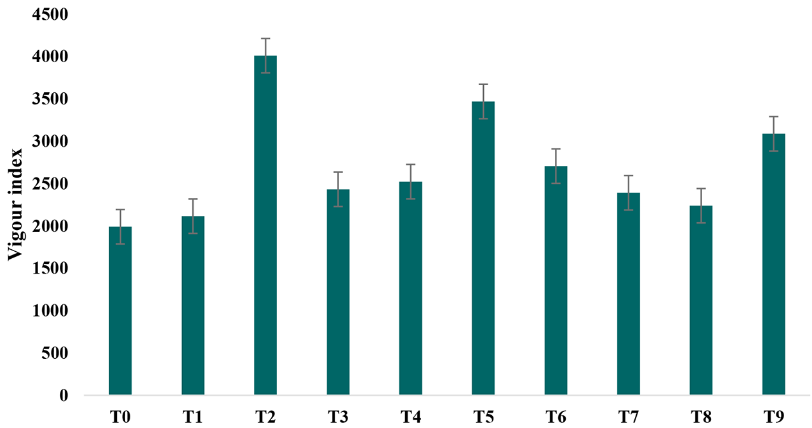



T0- Dry; T1- Water soaked; T2- Humic acid; T3- Chitosan; T4- Kelp extract (Red); T5- Kelp extract (Brown); T6- Moringa leaf extract; T7- Neem leaf extract; T8- Rhizobium; T9- *Pseudomonas fluorescens*

**Fig. 1.** Effect of biostimulants on seed germination percentage in cowpea var VBN 4.

studies and taken up for further investigation. Notable variations were noted between the treatments, wherein the seeds were treated with 5% humic acid for 8h have recorded 98% germination, root length (11.7 cm), shoot length (29.2 cm) and vigor index (4008) followed by kelp extract brown and *Pseudomonas fluorescens.* The water-soaked seeds recorded 82%, 10.2 cm, 15.6 cm and 2114 for germination, root length, shoot length and vigour index respectively (Figs. 1–3).

Applying humic acid for seed treatment greatly accelerated the growth of the roots and shoots. Highest root growth results from the rhizogenic action of humic acid, which significantly increases the rootlets endogenously (Hartwigsen and Evans




T0- Dry; T1- Water soaked; T2- Humic acid; T3- Chitosan; T4- Kelp extract (Red); T5- Kelp extract (Brown); T6- Moringa leaf extract; T7- Neem leaf extract; T8- Rhizobium; T9- *Pseudomonas fluorescens*

**Fig. 2.** Effect of biostimulants on seedling vigor in cowpea var VBN 4.

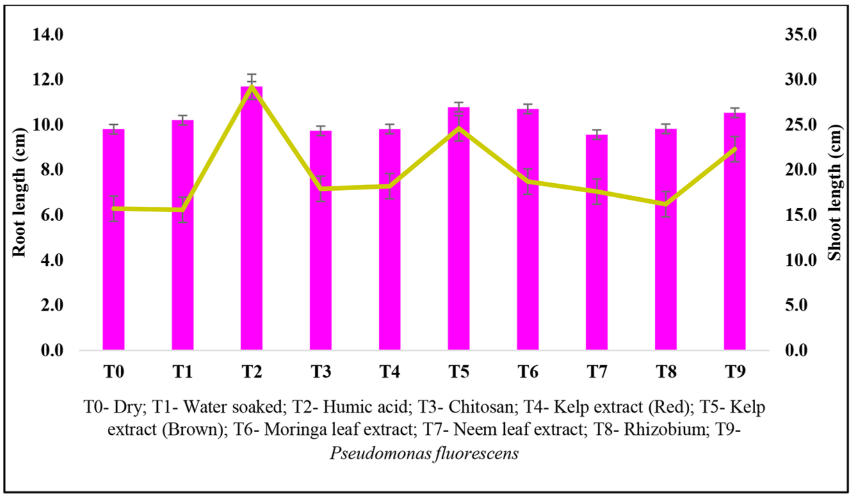



**Fig. 3.** Effect of biostimulants on root length and shoot length in cowpea var VBN 4.

2000). Conversely, elevated HA concentrations either inhibited plant growth or decreased the amount of nutrients in the plant. Their ability to chelate and their hormone-like activity can both contribute to the explanation of this seemingly perplexing anomaly (Onder Turkmen *et al*. 2004). This makes sense given how complex and varied humic acid is. We believe that humic substances are a significant factor in the vigour of seedlings. Our results are similar to Mohammed Basahi 2021, Yang *et al.* 2023 and Poomani *et al.* 2023.

Following humic acid seed treatment, the kelp extract demonstrates the second-highest values of seedling characteristics. Numerous compounds, including antimicrobial compounds, phytohormones, lipids, carbohydrates, proteins, amino acids, and osmoprotectants, that may be used as growth sup-

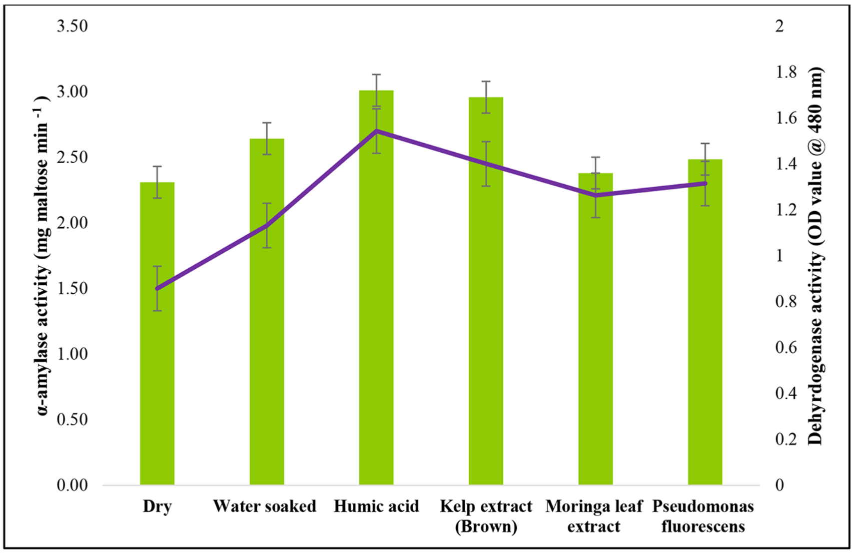



**Fig. 4.** Effect of biostimulants on α-amylase activity and dehydrogenase activity in cowpea var VBN 4.

plements are primarily found in kelp extracts. It has enhanced the germination values and seedling growth of this particular crop because it contains plant growth hormones like gibberellins, cytokinins, and auxins (Sharma *et al*. 2014, Battacharyya *et al.* 2015, Nabti *et al.* 2016, Carvalho *et al.* 2013). To increase seed germination, crop growth and development, seaweed extracts have been applied as a seed treatment (Elavarasan *et al.* 2021). Similar findings were reported by Nguyen Quang Thinh and Sundareswaran (2019), Mohammed *et al*. (2023).

Thirdly, the highest values of physiological characters in cowpea seeds were recorded by the PGPR. Indole acetic acid (IAA) is a common natural plant hormone that helps in root growth. Many rhizobacteria, which colonize seed or root surfaces, can produce IAA. When combined with the plant's own IAA, it promotes cell growth and improves the plant's ability to absorb minerals and nutrients from the soil. This ability is believed to be present in up to 80% of rhizobacteria (Govind *et al.* 2015, Maldonado *et al.* 2020). *P. fluorescens* AF8I1 has a high solubility activity for potassium and is capable of solubilizing phosphate (Fiodor *et al.* 2023). Furthermore, high IAA hormone synthesis and ACC deaminase activity. Our results coincide with Yousaf *et al.* (2019), Irfan Erdemci (2020), Valluri *et al.* (2021) and Singh *et al.* (2023).

The enzyme activities of dehydrogenase and α-amylase were recorded higher in humic acid 5% at 8 hours, followed by kelp extract (brown) and *Pseudomonas fluorescens* treated seeds (Fig. 4). Natural plant growth hormones such as auxins and cytokinins found in humic acids stimulate root growth and seed germination, leading to healthy seedlings (Lee and Bartlett 1976). Humic substances act as enzyme activators, increasing the activity of several enzymes involved in metabolic processes critical for seed germination and early seedling growth, while maintaining the correct electrolyte balance necessary for germination (Nardi *et al.* 2002). Similarly, cytokinins in brown seaweeds stimulate cell division and growth, promoting early seedling development and germination (Craigie 2011). Brown seaweeds also contain laminarin, a polysaccharide that enhances seed germination by accelerating seed metabolism and providing an energy source for early growth. By supplying essential nutrients, hormones, antioxidants, and stress tolerance mechanisms, these biochemical components of brown seaweeds work together to improve seed germination and seedling vigor.

The biostimulants that we employed in this investigation contain a significant amount of plant hormones. These hormones possess the capacity to impact the biochemical mechanisms within seeds. α-amylase and dehydrogenase are more potent enzymes that play a major role in determining the viability and liveliness of seeds (Fathaunnisha *et al.* 2019, Weerasekara *et al.* 2021). Thus, seeds treated with biostimulants will demonstrate their stability and growth.

From this research, we conclude that cowpea seeds soaked in the biostimulants of Humic acid @5% for 8 hours registered higher seedling vigor followed by brown kelp extract and *Pseudomonas fluorescens.* The biostimulants treated seeds increased the seedling vigor at a rate of 15% more than the water-soaked seeds. Overall, this study has demonstrated that biostimulants derived from natural sources are less expensive and can stabilize plant growth.

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### REFERENCES

Bagateli JR, Dorr CS, Schuch LOB, Meneghello GE (2019) Productive performance of soybean plants originated from seed lots with increasing vigor levels. *Journal of Seed Science* 41: 151–159. https://doi.org/10.1590/2317-1545v41n2199320.

Battacharyya D, Babgohari MZ, Rathor P, Prithiviraj B (2015) Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae* 196: 39–48. http://dx.doi.org/10.1016/j.scienta.2015.09.012.

Carvalho MEA, Castro PRC, Novembre ADC, Chamma H (2013) Seaweed extract improves the vigor and provides the rapid emergence of dry bean seeds. *Am J Agric Environmental Sci-*

*ence & Technology* 13: 1104–1107. DOI:10.5281/zenodo.51608.

Colla G, Rouphael Y (2015) Biostimulants in horticulture. *Scientia Horticulturae* 96 : 1–2. DOI:10.1016/j.scienta.2015.10.044.

Craigie J (2011) Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology* 23(3): 371-393. DOI:10.1007/s10811-010-9560-4.

Elavarasan E, Padmavathi S, Satheeshkumar P, Suganthi S, Kamaraj A (2021) Role of seaweed extract application on physiological planting value of rice (*Oryza sativa* L.). *International Journal of Botany Studies* 6 (2) ;172–174.

EU (2019) Regulation of the european parliament and of the council laying down rules on the making available on the market of EU fertilizing products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003.

Fan Yang, Yue Yuan, Qingyu Liu, Xi Zhang, Shuang Gai, Yongxu Jin, Kui Cheng (2023) Artificial humic acid promotes growth of maize seedling under alkali conditions. *Journal of Environmental pollution* 327: 121588. DOI: 10.1016/j.envpol.2023.121588

Fathaunnisha S, Subramani Nagaraj, Hemamalini V (2019) Influence of Seaweed Liquid Fertilizer on Growth and Biochemical Parameters of *Vigna mungo* and *Vigna radiata. Journal of Emerging Technologies and Innovative Research* 6 (6): 497-507.

Fiodor A, Ajijah N, Dziewit L, Pranaw K (2023) Biopriming of seed with plant growth-promoting bacteria for improved germination and seedling growth. *Frontiers in microbiology* 14:1142966. doi: 10.3389/fmicb.2023.1142966.

Govind G, Parihar SS, Ahirwar NK, Snehi SK, Vinod S (2015) Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture . *Journal of Microbial and Biochemical Technology* 7: 96–102. DOI: 10.4172/1948-5948.1000188.

Hartwigsen JA, Evans MR (2000) Humic acids seed and substrate treatments promote seedling root development. *Hort Science* 35: 1231–1233. DOI:10.21273/HORTSCI.35.7.1231.

Irfan Erdemci (2020) Effect of Pseudomonas Fluorescent Rhizo-bacteria on Growth and Seed Quality in Lentil (*Lens culinaris* Medik.). *Communications in Soil Science and Plant Analysis* 51(14): 1852–1858. https://doi.org/10.1080/00103624.2020.1798987.

ISTA (1999) International rules for seed testing. *Seed Science and Technology, Supplement Rules* 13: 209-355.

Kittock DA. Law (1968) Relationship of seedling vigor to respiration and Tetrazolium Chloride reduction by Germinating Wheat Seeds1. *Agronomy Journal* 60 (3) : 286-288. https://doi.org/10.2134/agronj1968.00021962006000030012x.

Lee YS, Bartlett RJ (1976) Stimulation of plant growth by humic substances. *Soil Science Society of America Journal* 40; 876-879. https://doi.org/10.2136/sssaj1976.03615995004000060023x.

Maldonado S, Rodrícguez A, Ávila B, Morales P, González MP, Araya Angel JP A (2020) Enhanced crop productivity and sustainability by using native phosphate solubilizing rhizobacteria in the agriculture of arid zones. *Frontiers in Sustainable Food Systems* 4: 607355. DOI:10.3389/fsufs.2020.607355.

Mohammed Basahi (2023) Humic acid improved germination rate, seedling growth and antioxidant system of pea (*Pisum sativum* L. var. Alicia) grown in water polluted with $CdCl_2$. *AIMS Environmental Science* 8 (4); 358–370. doi: 10.3934/environsci.2021023.

Mohammed Soha, Mostafa M, El-Sheekh, Saadia Hamed Aly, Maha Al-Harbi, Amr Elkelish, Aziza Nagah (2023) Inductive role of the brown alga *Sargassum polycystum* on growth and biosynthesis of imperative metabolites and antioxidants of two crop plants". *Frontiers in Plant Science* 14: 1136325. https://doi.org/10.3389/fpls.2023.1136325.

Nabti E, Jha B, Hartmann A (2016) Impact of seaweeds on agricultural crop production as biofertilizer. *International Journal of Environmental Science and Technology* 14: 1119–1134. DOI 10.1007/s13762-016-1202-1.

Nardi S, Pizzeghello D, Muscolo A, Vianello A (2002) Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry* 34 : 1527-1536. http://dx.doi.org/10.1016/S0038-0717(02)00174-8.

Nguyen Quang Thinh, Sundareswaran S (2019) Effect of seed priming with seaweed extracts on seed quality parameters in blackgram (*Vigna mungo* L.) cv CO 6". *International Journal of Chemical Studies* 7(3): 813-817.

Onder Turkmen, Atilla Dursun, Metin Turan, Çeknas Erdinç (2004) Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (*Lycopersicon esculentum* L.) seedlings under saline soil conditions. *Acta Agriculturae Scandinavica, Section B -Soil and Plant Science* 54(3): 168-174. https://doi.org/10.1080/09064710310022014.

Paul AK, Mukh S, Sircar SM (1970) Metabolic changes in rice seeds during storage. *Indian Journal of Agricultural Sciences* 40 (12) : 1031-1036.

Saima Yousaf, Ali Zohaib, Shakeel Ahmad Anjum, Tahira Tabassum, Tasawer Abbas, Sohail Irshad, Usman Javed, Naila Farooq (2019) Effect of seed inoculation with plant growth promoting Rhizobacteria on yield and quality of soybean. *Pakistan Journal of Agricultural Research* 32 (1) : 177-184. http://dx.doi.org/10.17582/journal.pjar/2019/32.1.

Sefa-Dedeh S, Stanley DW (1979) The relationship of microstructure of cowpeas to water absorption and dehulling properties. *Cereal Chemists* 56(4): 379–386.

Sharma HSS, Fleming CC, Selby C, Rao JR, Martin T (2014) Plant biostimulants: A review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *Journal of Applied Phycology* 26 : 465–490. DOI 10.1007/s10811-013-0101-9 .

Sindhu M, Kumar A, Yadav H, Chaudhary D, Jaiwal R, Jaiwal PK (2019) Current advances and future directions in genetic enhancement of a climate resilient food legume crop, cowpea (*Vigna unguiculata* L. Walp.). *Plant Cell, Tissue and Organ Culture* 139: 429–453. https://doi.org/10.1007/s11240-019-01695-3.

Singh Alok, Rawat Deepa, Gautam Rajani, Yadav Shikha (2023) Effect of seed bio-priming with cold tolerant *Pseudomonas fluorescens* and soil application of cow urine on germination, growth and nutrient uptake in pea. *Journal of Agriculture, Biology and Applied Statistics* 2(1): 1-9. https://DOI:10.47509/JABAS.2023.v02i01.01.

Subramanian Poomani, Yadav Sangita, Choudhary Ravish, Singh Dharmendra, Dahuja Anil, Yadav Shiv Kumar (2023) Seed priming with humic acid modifies seedling vigor and biochemical response of lentil under heat stress conditions". *Turkish Journal of Agriculture and Forestry* 47: 1043-1057. Doi:10.55730/1300-011X.3147.

Valluri Atchyuth, Chaurasia Arun Kumar, Jagadam Reddy Kiran (2021) Impact of organic and inorganic seed priming methods on growth and yield attributes in lentil (*Lens culinaris* L.). *The Pharma Innovation Journal* 10(4): 1063-1065.

Weerasekara I, Sinniah UR, Namasivayam P, Nazli MH, Abdurahman SA, Ghazali MN (2021) Priming with humic acid to reverse ageing damage in soybean [*Glycine max* (L.) Merrill.] seeds. *Agriculture* 11 (10) : 966, 1-18. https://doi.org/10.3390/agriculture11100966.