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Clinical Review

Efficacy of Aloe-Booster Containing ALM Green for the Replant Failure of *Rubia akane, Persicaria tinctoria,* and *Allium cepa* on Dried Fields in Circulation Type-Agriculture: Case Report 1-3

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Abstract

Efficacy of Aloe-booster containing ALM (Aqua Life Modifier) green, the official Kampo-plant roots grown regulator, ALM, for the replant failure of *Rubia akane*, *Persicaria tinctoria* and *Allium cepa*, was demonstrated. Inhibition effects of the replant failure with the endophytic fungus of Aloe-booster were shown for *R. akane*, *P. tinctoria* and *A. cepa*. The effects of Aloe-booster in the dried cultivation fields were widely expressed. Potential efficacy of endophytic microbiota, *Trichoderma* spp. in Aloe vera, against the plant pathogens such as *Fusarium* spp. as an Aloe-booster, one of bio-stimulants, for replant failure was strongly expected in circulation type-agriculture.

Keywords: *Efficacy; Aloe-Booster; ALM Green; Replant Failure; Rubia akane; Persicaria tinctoria; Allium cepa*

Introduction

The severe unusual weather we are experiencing could be a result of global warming and it continues every year, and we are experiencing could be a result of global warming and we have to save these severe weather conditions specially in the agriculture fields. In recent years, bio-stimulants' names sparked an interest with many crop products with these products getting attention. We found this is much to debate on their effectiveness. Bio-stimulants as "materials that, in minute quantities" for describing bio-stimulants from nutrients and soil amendments, which also promote plant growth but are applied in larger quantities. The world is currently facing the combined challenges of feeding a growing population whilst also protecting the environment and producing renewable sources of energy [1]. Demand for food is expected to increase 2~5 fold by 2030 and food production is predicted to increase by 60% in the coming decades to meet these demands. The new challenge is to build systems of food production based on alternative intensification strategies which promote nutrient-use efficacy, reduce the need for disease and pest control, increase water-use efficiency and conservation which restores solid fertility [2].

Short chain fatty acids as bio-stimulants for plants

Short chain fatty acids (SCFAs) can be used as bio-stimulants for plants. SCFAs such as acetic, propionic and butyric acids have been shown to promote plant growth and development. These compounds can be produced by bacteria in the soil and are also found in some plant-based products, such as endophytic microbiota. The research is amid at developing new biologic formulations based on the combination of small microbial consortia containing two *Trichoderma* spp. with a medium-long chain fatty acid mixture (FAm). The bioactivity of these formulations was investigated on different vegetable crops in terms of biocontrol, growth promotion, yield and quality improvements. FAm application reduced *Botrytis cinerea* necrosis by up to 90% compared to the impacted control plants and some of the assayed *Trichoderma* spp. + FAm combinations contained *Rhizoctonia* disease, reaching more than 90% reduction of tomato and lettuce plant mortality. An increasing yield, ranging to 25 and 90%, was recorded on treated tomato, lettuce and kohlrabi; compared to untreated plants. A significant enrichment in carotenoids (+60%) and glucosinolates (+39%) was measured on biotreated plants compared to controls [3].

Acetic acid: A cheap but chief metabolic regulator for abiotic stress tolerance in plants

Plants constantly face a variety of abiotic stresses, such as drought, salinity and metalloid toxicity, all of which possess significant threats to plant growth and yield potential. Improving plant resilience to such abiotic stresses bears paramount important metabolites with multifaceted roles in regulating plant adaptation to diverse abiotic stresses [4].

The simple and universal function of acetic acid to overcome the drought crisis plays an essential role in conferring tolerance to water deficit stress in plants. T. Kudo., *et al*. highlighted the function of acetic acid in conferring tolerance to water deficit stress [5].

Role of bio-stimulants in the mitigation of abiotic stress

Abiotic pressures are occurring more frequently in light of the context of climate change. It has been claimed that bio-stimulants are essential for change. It has been claimed that bio-stimulants are reducing these stresses. The role of bio-stimulants is improving nutritional quality.

Crop produce quality is a vital aspect related to food security. By enhancing the produce quality, several human nutritional issues can be alleviated, even with the currently available amount of food [6].

In our current review article, Yagi., *et al*. presented "Bio-stimulant effects of the fermentation extracted by endophytic microbiota from outer leaf layer in Aloe vera". This is what we want to tell the people with Aloe vera in business and agriculture. Bio-stimulants which were recently formulated as the 3rd pesticides in Japan, positioned as one of the natural fertilization products applied as an organic Aloe-booster for agricultural suppliers. Aloe-booster is composed of the fermented extract from the outer layer of Aloe vera, with the fermented extract of twelve Kampo-drugs (ALM green: Aqua Life Modifier) [7].

The plants under drought stress treated with the bio-stimulant product that was formulated with botanical extracts and treated to increase the tolerance of cultivated plants to drought, that sought to establish the action of said bio-stimulant. The plants under drought stress treated with the bio-stimulants showed an increase in glucosinolates, conjugated polyamines and phytoalexins [8].

Limitation in the use of bio-stimulants in sustainable agriculture

We will not only highlight the potential use of bio-stimulants to face future agricultural changes, but also take a critical look at their limitations, underlining the importance of a broad vision of sustainable agriculture in the context of recent severe climate-up-changes. Global warming is primarily caused by increased greenhouse gas emission from human activities and release of carbon dioxide from the burning fossil fuels.

Classification of *Rubia spp*.

Rubia spp. in general country are mainly classified as Japan, China, UK and India as follows: Japanese *Rubia* spp.: *Rubia akane*, *R. argyi*, *R. cordifolia*, China *Rubia* spp.: *Rubia cordifolia*, Mudder: *Rubia tinctorum*, and Indian: *Rubia cordifolia*.

Establishment of Japan Red Project

One of Japan's red-vegetable dye with *Rubia akane* roots, has been getting familiar with Japanese and has been safely kept as the colors-culture in Japanese. To keep *R. akane* safe, "Japan Red Project" was established to the development of the colors-culture and revival for its industrialization in 2009. The red color from *R. akane* roots was widely used for the colors-culture in Japan, and are applied from the following anthraquinones: alizarin, purpurin and munjistin. Akane dye comes from the roots of the plant. Akane is not as commonly grown in Japanese anymore but it is still widely formed in India which is where it comes from. For a limited time from December to March, you can make some lovely red dyed material using Akane or Red madder. In addition to the traditional patterns of Omasu and Tachiwaku, there are over 800 unique designers. The squeezing is done by hand by dozens of local squeezers. One of the charms is the warm beauty and unique beauty of handcrafts, and the color changes that are becoming more and more delicious every year.

Case report 1: Application of Aloe-booster (the mixture from the fermented extract of Aloe vera dry-rind powders and ALM green) to cultivation of *Rubia akane* (Rubiaceae) was widely performed by circulation-type agriculture at 200 - 400 meter Hight, in Ikeda Town, Miyoshi City, Tokushima prefecture, Japan, in May, 2025.

Case report 2: Application of Aloe-booster to cultivation of *Persicaria tinctoria* (Polygonaceae) Aizumi Town in Tokushima prefecture in June, 2025.

Case report 3: Application of Aloe-booster to cultivation of *Allium cepa* (Amaryllidaceae) in Saku City, Nagano prefecture, in May 2024.

Cultivation: Effects for replant failure of *Rubia akane*, *Persicaria tinctoria*, and *Allium cepa* with the diluted Aloe-booster in dried cultivation fields.

Medical uses of *Rubia akane* roots

R. akane roots extract as herbal medicines were reported for circulating systems: hemostatic agent, vasodilators, and a typical genital bleeding.

In ancient times, *Rubia* spp. plants were used in Chinese medicine to treat rheumatoid arthritis. Research studies have proven that purpurin exerts antimicrobial, neuro modulatory, antigenotoxic, anti-inflammatory, anticancer and antioxidant effects. The investigation was proposed to evaluate the efficacy of purpurin against myocardial infarction in rat [9].

The effect of purpurin on inflammation was investigated using macrophage RAW 2647 inflammatory cells, induced by lipopolysaccharide, and adjuvant-induced arthritis rats were established to explore the effect of pharmacology and molecular docking were integrated to dig out the prospective target. Purpurin showed a significant anti-inflammatory effect by reducing the content of IL-6, TNF-α, IL-1ß and increasing IL-10. The result of the study strongly indicated that purpurin has a potential effect on anti-rheumatoid arthritis [10].

Alizarin from *R. cordifolia* has a variety of pharmacological effects, including anti-oxidant, anti-inflammatory and anti-platelet. Alizarin not only restored systolic blood pressure (SBP) and diastolic blood pressure. Alizarin not only restored SBP and increased endothelium dependent relation in spontaneously hypertensive rats, but also inhibited oxidation stress-induced mitochondrial damage and also significantly delayed the vascular senescence effect in hypertension, and the mechanism may be related to the activation of VEGFR2/eNOS signaling pathway [11].

Simultaneous determination of purpurin, munjistin and mulligan in rat's plasma was studied. Pharmacokinetic parameters of the three analytes showed a process of a slow absorption and metabolism after oral administration of *Rubia cordifolia* extract to rats [12].

Medical and clinical uses of *Persicaria tinctoria (Polygonum)*

Recent studies in chromosome-level genome assembly of the tetraploid medical and natural *P. tinctoria* were reported [13].

Recent research for tryptanthrin synthesis and medicinal perspectives

Tryptanthrin, one of the pyrrole-indole-quinazoline alkaloids in *P. tinctoria*, is a natural alkaloid containing anticancer, anti-inflammatory, anti-protozoal and anti-allergic activities. Recently, tryptanthrin demonstrated strong activity against for one of atopy disease fungi, *Malassezia furfur* [14] and *in vivo* test using mice of tryptanthrin showed anti-*Helicobacter pylori* and the improvement of hyperlipidemia effect were confirmed. In clinical studies the therapeutic effect on periodontal disease and candidiasis were confirmed [15]. Targeted discovery of anthranilic acid-based alkaloids with neuroprotective and acetylcholine esterase inhibitory effects from natural indigo plant (*P. tinctoria*) using DeepSAT technology were reported [16].

Sunshine in a bottle

Persicaria tinctoria extract offers a multitude of benefits in skin care formulation including tanning activity mood-enhancement and Vitamin D3 stimulation. By mimicking skin health, incorporating *P. tinctoria* extract into skincare products offers a promising avenue for addressing various skin concerns while also supporting holistic health. *Persicaria tinctoria* extract offers myriads of sun-mimicking benefits including tanning activity, mood enhancement and vitamin D3 stimulation. This can help address vitamin D3 deficiency and support overall skin and body health, particularly in regions and seasons with limited sunlight [17].

Application of Aloe-booster for Replant failure to *Rubia akane,* in Ikeda town, Tokushima prefecture, *Persicaria tinctoria,* in Aizumi town, Tokushima prefecture, *Allium cepa* in Saku City, Nagano prefecture

As one of the resolving problems from continuous cropping, soil improvement, the cropping rotation is proposed. We used the fermentation extract from Aloe vera outer-leaf, Aloe-booster, in replant failure. Case report 1-3 showed the effect for application of Aloe-booster, as one of bio-stimulants for three test samples.

Effects of Aloe-booster for replant failure

As pathogenic fungi for replant failure, *Fusarium* and *Rhizoctonia* microbiota are the main sources, and *Trichoderma* fungus is one of the anti-fungi groups which is down the activation under pH 8 over. A comparative study of different temperature and pH for the control of *Rhizoctonia solani* is following. The growth of *Rhizoctonia solani* showed the greatest at 25℃ and pH 5.5, compared with tested following Temperature (℃): 10, 15, 25, 35 and pH: 5.5, 7.0, 8.5. Biological functions of *Trichoderma* spp. for agricultural applications were reported [18].

Stimulation with *Trichoderma* spp. Endophytic fungus, from Aloe vera for agricultural crops

Trichoderma fungus stimulated the growth of the crop roots expressed by following biological worldwide research [19].

Several damages were caused with *Rhizoctonia solani* in the potato crop and the current limitations for its control justify the assessment of potential biocontrol agents and their relationship with abiotic factors to be successfully included in sustainable production systems. A greater ability to compete for the substrate is observed, enhanced by its higher growth rate. Hyphal interaction mechanisms were varied and, at least, 92% of the isolations showed a minimum of two different types. Knowledge of the behaviors of the different strains in front of varied abiotic factors will enable an understanding of the population dynamic of *Trichoderma* and the identification of the

most efficient strains for the control of *R. solani*. *Trichoderma* spp. has been widely used in agricultural applications due to its well-known biological control mechanism. The usage of this microbial inoculant in *Trichoderma*-based products attracted the attention of researchers to discover more on other potential benefits of *Trichoderma* spp. Hence, through research work from worldwide researchers to discover more on other potential benefits of *Trichoderma* spp. Hence through research work from worldwide researchers, the authors presented the success of *Trichoderma* spp. relate to present disease, plant disease, plant growth, decomposition process and bioremediation. Plus, their secondary metabolites production in agroecosystem will also be reviewed in this paper. These surprising findings bring enormous advantages to the agriculture industry in order to apply environmentally friendly agriculture practices [19].

Trichoderma harzianum had the most effective results on features of height, number of leaves, and off shoots of Aloe vera plant. The significant increase was observed in the plant height, number of Aloe vera leaves and off shoots that were treated with *Trichoderma harzianum* compared with the control [20].

Inhibition spectrum and antagonistic mechanism of an endophytic fungus *Trichoderma harzianum* LH-7, isolated from a medicinal plant Aloe vera were investigated by *in vitro* culture methods against 9 kinds of plant pathogens. The results show that nutrient composition and hyper parasitism were two primarily antagonist approaches that strain LH-7 adopted to inhibit the tested plant pathogens with a significant inhibition rate of 62.4% and 88.4%. Moreover, the active compound from metabolites of LH-7 could cause pathogen mycelial deformities, cell wall rupture and conidial malformation, leading to the effective inhibition on pathogens growth reproduction [21]. Biocontrol of *Fusarium* oxysporum by *Trichoderma* spp. in Aloe vera under greenhouse and field conditions was reported [22].

Case report 1: Application of Aloe vera booster for soil improvement and soil infection in cultivation of *Rubia akane* roots was examined in June, 2025

Akane roots were deeply rooted in growing just two weeks compared with the control groups. And the harvest amounts of the red-roots in *R. akane* increased about 10% to 20% [23].

Case report 2: Effects of Aloe-booster for replant failure of *Persicaria tinctoria* in agriculture fields

After the planting of *P. tinctoria* in the dried agricultural field, the diluted Aloe-booster was sprayed in the replant failure fields. The dark green leaves are grown in control plant leaves and were harvested.

Case report 3: Effects of Aloe-booster for replant failure of *Allium cepa* in agriculture fields

One of causative bacteria to *Allium cepa* is determined as *Fusarium oxysporum f. sp. cepa*.

The harvested *Allium cepa* showed clear white root area, while the non-treated *A. cepa* infected with pathogens showed a lot of black infected spot area.

Potential application of endophytic bacteria associated with Aloe vera

The study highlights the significance of endophytic bacteria associated with Aloe vera. Understanding the symbiotic relationship between Aloe vera and the endophytic bacteria, such as *Bacillus, Pseudomonas*, *Enterobacter*, and *Streptomyces* among other: *Trichoderma* [24], Hypocreaceae, and *Lactobacillus* [25], Lactobacillaceae, could contribute to advancement in biotechnology, agriculture and medicine. These endophytic bacteria in Aloe vera exhibit different functional traits that contribute to Aloe vera over all health and resilience. These microorganisms exhibit plant growth-promotion properties, such as production of phytohormones, nitrogen fixation, and phosphate solubilization, which enhance the plant's adaptation to various environmental conditions. They can be utilized as bio-stimulants, bio-fertilizers, and antimicrobial agents. Understanding the symbiotic relationship between Aloe vera and its endophytic bacteria could contribute to advancements in biotechnology, agriculture and medicine [26].

Conclusion

Application of Aloe-booster for *Rubia akane*-cultivation presented a good yield comparing the control groups in agricultural fields of 200 - 400 meter Hight, Miyoshi City, Tokushima prefecture in Japan. Bio-stimulants contained in Aloe-booster strongly expressed the efficacy for practical cultivation of herbal medicine, *Rubia akane* in agricultural fields, Miyoshi City, Tokushima prefecture in 2025. Replant failure of *Persicaria tinctoria* in Aizumi-Town, Tokushima prefecture and one of the replant failures for *Allium cepa* in Saku City, Nagano, prefecture was widely examined in 2025.

Inhibition and antagonistic mechanisms with endophytic microbial *Trichoderma* spp. from the fermentation extract of outer leaf Aloe vera could support an Aloe-booster function as one of bio-stimulants against plant pathogens such as *Fusarium* spp. in the replant failure.

Further Aloe-booster's functions as bio-stimulants for powdery mildew and the replant failure, such as *Cucumis sativa* (Cucurbitaceae), *Phaseolus vulgaris* (Leguminosae) and *Fragaria virginiana/F. chiloensis* (Rosaceae) are strongly expected in a daily vegetable supply.

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