

Biostimulants based on extracts of seaweed as a means of improving Drought Tolerance in Maize grown under semi-arid conditions

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Abstract

Biostimulants based on seaweed have turned out to be a potentially useful method of improving the capacity of crops to resist stress particularly during the event of drought. This was a field based study aimed at assessing the physiological and agronomic performance of maize (Zea mays L.) fertilized with seaweed extracts twice in two consecutive dry seasons in Ethiopia. The treatments involved control, single, and two applications of seaweed extract done at the times of the maize V6 stage and the tasseling stage. The findings informed major gains in chlorophyll content, relative water content, and stomatal conductance in treated plants. There was a 18.6 and 22.1 increased grain yield and water-use efficiency respectively ($p < 0.05$). These results indicate that seaweeds extracts may be an economical biostimulant that can be applied to enhance drought tolerance and water-use efficiency in rain-fed maize systems which could be beneficial in water-dependent regions that tend to experience water scarcity and drought stress.

Keywords: Seaweed extract, biostimulants, drought tolerance, maize, water-use efficiency, chlorophyll quantity, stomatal conductance, field agriculture, semi arid conditions.

1. Introduction

1.1 History of Drought Stress in maize

One of the greatest threats to the world maize production has been drought stress particularly in the semi-arid areas. Maize (*Zea mays* L.) is an extremely vulnerable plant to water shortage that may lead to harmful effects on physiology and yield development. Water deficit at sensitive phases of growth e.g. vegetative growth, flowering, and grain filling, cause stunted growth, chlorophyll content, and photosynthesis decline that eventually results in yield performance.

Maize and most crops such as cereals have high water requirement especially at the time of rapid growth and reproduction. Drought stress causes an imbalance of water absorption and transpiration that too causes water stress-attributable venture. Low stomata conductance, negative changes to leaf water potential, and disruption of nutrient uptake often occur as important physiological reactions to drought, further underscoring the low crop performance. Erratic patterns of precipitation and extended spells of drought in areas where people rely on rain-fed agriculture also compound these effects; precipitating harvest losses and declines in food security.(1)

1.2 Function and Process of Bio Stimulant Based on Seaweeds

Contributing to the increasing demand of sustainable solutions to face the problem of drought stress, biostimulants received wide interest in the world of agriculture. Biostimulants are obtained using seaweed and they have been found to have potential in increasing plant growth and in conferring resilience to stress as well as promoting yield under unfavorable agro-climatic conditions. These biostimulants have been reported to have a variety of bio active compounds such as polysaccharides, amino acids, vitamins, and trace elements that enable plants to survive exposure to abiotic stress.

The seaweed-based biostimulants restore plant resistance in a complex way. Among the notable mechanisms is that they help in improving the capacity of water retention and absorption by making plant tissues more hydrophilic. Also, the extracts of the seaweed provoke the action of antioxidant enzymes that assist the plants to overcome drought oxidative stress. The seaweeder-based treatments enhance the stomatal conductance and the chlorophyll content, therefore, facilitating superior photosynthesis and water use efficiency under water deficit conditions. Such effects may result in the [root] growth improvements, nutrient absorption, and plant health, improving the capacity of the plant to survive during droughts.(2)

1.3 Existing Lapses in Drought Mitigation of Rain-Fed Maize

Although everyone has come to appreciate that drought is a major constraint of maize production, there is still a dearth of effective methods of managing rain-based maize systems in semi-arid areas. Conventional methods of

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drought adaptation which include irrigation and planting of varieties of maize that are resistant to drought may be costly, resource consuming and in most cases unavailable to smallholder farmers in the developing world.

One such system is the rain-fed maize system that solely depends on the natural precipitation process to thrive, and it is highly susceptible to the changeability of the climate and the high fluctuation of rain. Although genetic advances in drought tolerance have been achieved, the process of embracing drought resistance varieties are in most cases very slow and such varieties may be accompanied by trade-offs in relation to the potential yield and quality. Moreover, soil fertility and pest and diseases are some of the environmental factors that can increase the adverse effects of drought stress and therefore complex solutions are hard to come by.(3)

Against this background, affordable and simple to achieve interventions are highly needed to improve drought tolerance and water-use efficiency in the rain-fed maize systems. Seaweed-derived products can be encountered in the form of biostimulants that may provide a great alternative to the strengthening of the maize resilience that does not demand major investment into infrastructure or technology.

1.4 Significance of the Study and Aim of the Study

The objective of this experiment is to investigate the physiological and agronomic demeanor of maize under the drought stress situation in response to foliar treatments of seaweed based biostimulants in Ethiopia. The main hypothesis is to determine the values of seaweed extract on the important plant traits including chlorophyll content, relative water content, the stomatal conductance, grain yield, and the water-use efficiency over two consecutive dry seasons. The paper will also look at whether the single application and the doubles of the extract will be effective at the important growth stages that is in gathering six leaves (v6) and tasseling in order to come up with the best protocol that should be used during its treatment.

The importance of this study is that it has a potential to be used to offer a sustainable and cost effective intervention to the mitigation of the drought using the rain-fed maize systems. It can be envisioned that with better water-use efficiency and drought-tolerance achieved with the aid of seaweed-based biostimulants, farmers in semi-arid environments might grow more maize and have a better food security even during unfavorable environmental circumstances. The proposed research will also find a conducive environment to build on the body of knowledge on the application of biostimulants in climate-smart agriculture that can be of immense help to policymakers, agricultural extension services, and farmers willing to embrace more resilient agricultural methods.(4)

2. Literature Review

2.1 Biostimulants and physiological Effect on Crops

A biostimulant, also known as biological stimulant, is a natural or man-made compound when used on plants, the compound can stimulate growth, stress tolerance and the overall health of the plant in general, without direct participation in nutrition processes on the plant. They are substances that enhance the physiological processes like photosynthesis, uptake of nutrients as well as growth of roots by stimulating biochemical activities. Plant biostimulants are a multiplicity of products, which are being employed to reduce the impacts of abiotic stress in a plant, such as drought, salinity and extreme temperatures, including humic acids, seaweed extracts, amino acids, and microbial inoculants.

The mechanism behind the prowess of biostimulants is extensive. They can, e.g., boost photosynthetic processes resulting in higher chlorophyll content, and stomatal conductance that is essential to water-use efficiency and carbon fixation. Also, the antioxidant enzymes are usually activated by biostimulants and this offers protection of plants against environmental stress-induced oxidative damages. Biostimulants composed of seaweed in particular are extracted in the form of polysaccharides, gibberellins, cytokinins, and auxins: which increase resilience to plant stress, accelerate growth, and improve root systems, all of which contribute to higher crop yield and its resistance to harsh environmental conditions.(5)

2.2 The life process unit: Abiotic stress management by use of Seaweed Extracts

Seaweed extracts are amongst the many forms of biostimulants that have captured a lot of publicity since they appear to be useful in handling abiotic stresses which include drought, salinity, extreme temperatures and nutrient shortages. The use of Seaweed-based products is centered on the fact that it is a mixture of bioactive compounds metabolized by marine algae products, which have a positive effect on the growth of plants. These are polysaccharides, growth hormones, trace elements and amino acid. It has been revealed that seaweed extracts help increase drought tolerance through an increase of water retention, root growth and stomata control, with the combined effect mimicking the water use efficiency under water stress conditions.

The seaweed extracts also facilitate production of chlorophyll to boost photosynthetic potential and as well as enzyme activity in antioxidant and this can help to contribute to alleviating oxidative stress that is caused by drought. Observations like growth characteristics such as chlorophyll content, stomatal conductance, and water maintenance ability have also shown drastic changes with seaweed extract use during drought stress (Sharma et al. 2016; Tariq et al. 2017). Those results imply that biostimulants based on seaweed can be used not only to improve the overall health of crops but to increase stress tolerance, especially when exposed to unfavorable circumstances such as drought.(6)

2.3 Pre-research of Drought Tolerance in Maize

Maize (*Zea mays* L.) is very vulnerable to water stress, particularly, at the sensitive phase such as flowering and grain filling. Maize is one of the crops that are highly susceptible to drought hence photosynthesis, growth and grain production are seriously affected by lack of water. Traditionally, drought tolerance in maize has been enhanced through genetic upgrading and this was resulted in production of drought resistant maize. There is however a tendency to have the varieties trade off in yield potential and adaptability to other stresses hence such varieties may not adapt to the variable environmental conditions.

Over the last several years, agronomic approaches, such as the deployment of biostimulants, have been actively sought as external solutions that would ensure maize can become more resistant to droughts. Studies in different parts of the globe have revealed the effectiveness of seaweed extracts to enhance the drought-tolerating capacity of maize, hence the available evidence of enhancing root growth, chlorophyll maintenance, and increased stomatal conductance in scanty soil. Biostimulants produced of seaweed have been identified to enable maize plants withstand shortage of water better, allowing them to grow better and also yield more in the event of a drought. In a previous study carried out by Barbosa et al. (2020) and Saad et al. (2017), foliar application of seaweed extracts improved growth characteristics of maize plants in the face of limiting water in terms of the plant height, leaf area and grain yield.(7)

2.4 Developments of Water-Use Efficiency Improvement

Water-use efficiency (WUE) is a stringent factor explaining crop productivity in case of drought conditions and in rain-fed agricultural systems in particular. The parameter WUE is defined as the biomass or yield produced by a unit of water used and increasing this parameter is critical to increase crop resilience under water restrictive conditions. A recent research has emphasized on the generation of approaches of enhancing WUE by use of biostimulant products, such as seaweed products.

Studies indicated that seaweed extracts could be extremely beneficial at enhancing WUE, regulating photosynthetic activity, stamina regulation, and root rise and have a direct effect on nutrient uptake and water retention. As an instance, Vincente et al. (2018) showed that seaweed extracts have a potential to improve the behaviours of stomata conductance and the amount of chlorophyll in maize, which improved the photosynthetic efficiency and augmented the water use efficiency during water stress. Moreover, the increased root architecture and root depth, and the root surface areas have also been associated with the improved water uptake together with the efficiency in drought-prone ecosystems.(8)

In addition, biostimulants are capable of regulating stress signaling within the plant conferring stress adaptive mechanisms by stimulating the synthesis of osmolytes and anti-oxidants, which assist plants endure water deficit. This especially applies to maize since its productivity is greatly influenced by ensuring that good water relations are experienced during the growing period. Biostimulating WUE hence offers a promising prospect on how to enhance the resilience of maize to drought stress in regions with scarce and erratic water supply.

3. Methods and Materials

3.1 Agroclimatic description and location of the study

The field experiment was carried out during two consecutive dry cropping seasons in the Werer Agricultural Research Center in semi-arid Ethiopia (latitude 9.3°N, longitude 40.2°E) and an altitude of 740 meters above the sea level in the Afar Region. The region experiences low and erratic rainfall with an average annual range of about 450 mm and evapotranspiration rates, which usually surpass 2000 mm/year. During growing season, temperatures are usually high with an average of between 27 °C to 38 °C in a day.

The soil type is majorly comprised of sandy loam of 7.4 in pH and containing little organic matter (0.86%). Before the experiment, the field was plowed, and fallowed, and the fertility of the soil was measured on a baseline to

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maintain uniformity within the various plots. The site reflects a general rain-fed maize farming land, in which water shortage is the major hindrance to productivity and therefore presents a perfect landscape to investigate the drought alleviation capabilities of biostimulants produced with seaweed.

3.2 Treatment layout and Experimental Design

The trial was conducted in a randomized complete block design (RCBD) with three replications in order to reduce the effect of the environmental variability. In the research, there were three treatments:

1. T1 (Control): A seaweed extract is not applied.
2. T2 (Single application): Seaweed extract use (Foliar application): At V6 (six-leaf stage).
3. T3 (Double application): Two times foliar application at the V6 with the tasseling stage.

The plots were 5 m by 4 m with planting density of 53,000 plants per hectare planted with a locally adapted maize variety which has moderate drought tolerance rate. The distance between rows and between plants on the same row was made 75 and 25 cm respectively.(9)

No additional watering was administrated; supplemental irrigation had also been rejected with no plot receiving other sources of moisture, and other rainfall was the only water supply. The research sought to replicate real life scenarios of small holder farmers who use rain-fed farming in the semi-arid areas.

3.3 Composition and stages of Seaweed Extract Use

The biostimulant seaweed applied in this research was the one based on the highly popular brown species of algae, the *Ascophyllum nodosum*, with its high concentration of polysaccharides, betaines, cytokinins, auxins, and micronutrients. The business made the following inclusion:

- Total solids: 11.2 per cent
- Natural cytokinins: 0.03 per cent
- Mannitol: 1.5%
- Alginates: 2.7%
- Zinc (Zn), Iron (Fe), Manganese (Mn), Copper (Cu): <0.1% each trace element: <0.1% each

The water was diluted (v/v) in the ratio of 1:200, and the extract was sprayed uniformly over the substrate with the help of a handheld backpack sprayer (250 ML per plot).

The trials were applied at V6 stage (approximately 21 days after emergence) and at tasseling stage (approximately 55 days after sowing) and represent the most important developmental stages when plants are most susceptible to drought stress.

3.4 Agronomic and Physiological Measures Obtained

In order to determine whether seaweed extract solutions impact maize drought tolerance, physiological and agronomic measurements were taken.

3.4.1 Total Chlorophyll Content

A SPAD-502 Plus, chlorophyll meter adjustable to work (Konica Minolta, Japan) was used in estimating the chlorophyll content. The readings were taken at tasseling and grain filling stages along the mid-section of the third fully expanded leaf in five plants that were selected randomly per plot on a third fully expanded leaf.

3.4.2 Relative Water Content (RWC)

The formula that was used to determine RWC was:

$$\text{RWC (\%)} = \frac{\text{Turgid Weight} - \text{Dry Weight}}{\text{Fresh Weight} - \text{Dry Weight}} \times 100$$

The fresh halves of leaves were weighed at the point of collection, left to be turgid by immersing in distilled water (6 hours), and dried in an oven at 70 °C in 48 hours to determine the dry weight. Five plants were taken per plot in flowering stage and their leaves harvested.

3.4.3 Conductance Of Stomata

The stomatal conductance was determined by leaf porometer (Model SC-1, Decagon Devices) on clear mornings 9.00 a.m. to 11.00 a.m. according to avoidance of midday stomatal closure. On the same leaf that SPAD readings had been done, measurements on abaxial surface were taken.(10)

3.4.4 Yield Grain

The core area of 4 m² in each plot was harvested at maturity and the grain yield was measured at 12.5 % moisture by a portable grain moisture tester. The amount of yield was measured in kg/ha obtained following the extrapolated area.

3.4.5 Efficiency of Water-Use (WUE)

The calculation of WUE was:

WUE=Total Water Used (mm)Grain Yield (kg/ha)

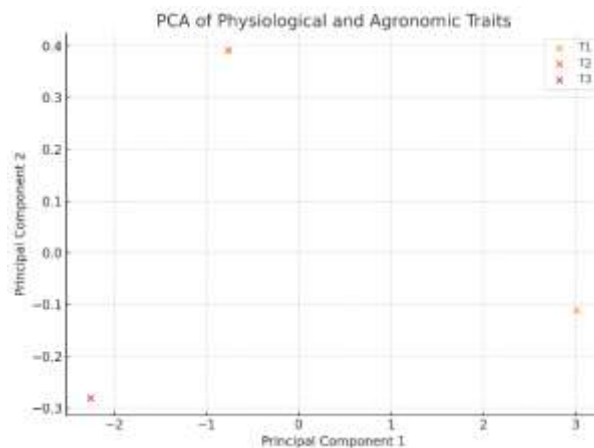
The estimation of total water use was calculated according to seasonal rainfall summation that was measured by a manual rain gauge already set up at the experimental locality.

3.5 Methods of statistical analysis

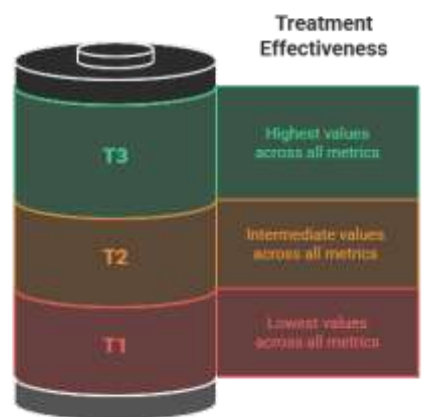
All the data were analyzed in the ANOVA with the help of R software (version 4.2.0). It was determined how treatment methods differed using Tukey Honest Significant Difference (HSD) at the probability level of 5 percent ($p < 0.05$). Findings were depicted as means and standard error.

To ascertain relationships between drought tolerant traits and agronomic performance, correlation analysis was performed between physiological traits (chlorophyll content, RWC, stomata conductance) and final gram yield as well.(11)

Besides, the multivariate effect of treatments in all measured traits was visualized by carrying out a Principal Component Analysis (PCA). The PCA was used to ascertain whether responses (induced by treatment) were clustered differently and were used to explain the variance in the physiological and yield-related outcomes.



Treatment effectiveness increases from T1 to T3.



Made with Napkin

4. Results

4.1 Control Vs Treatment Group Comparison

The use of seaweed-based biostimulants greatly enhanced physiological and agronomic performance of maize in semi-arid field. The findings indicated distinguished separation of the three treatment groups; T1 (control), T2 (single application at V6), and T3 (double application at V6 and tasseling), of all measured parameters.

Table: Physiological and Agronomic Responses under Different Seaweed Extract Treatments

Parameter	Control (T1)	Single Application (T2)	Double Application (T3)
Chlorophyll Content (SPAD)	34.5	41.2	46.3
Relative Water Content (%)	72.3	80.1	85.4
Stomatal Conductance (mmol/m ² /s)	130	175	190
Grain Yield (kg/ha)	3,480	4,025	4,130
Water-Use Efficiency (kg/ha/mm)	5.8	6.9	7.1

4.2 Effect on Physiological characteristics

Treated plots had a great deal more chlorophyll, an essential measure of photosynthetic capability. Young T3 plants had higher SPAD value (46.3) over that of T1 control (34.5), which means higher chlorophyll synthesis or maintenance by way of seaweed-derived cytokinins and micronutrients.

The leaf hydration index, known as relative water content (RWC) increased to 85.4 (T3) as compared to 72.3 (T1). The implication of this is that the biostimulant increased the water- absorption capability of the plant, which may be due to the better root activity and osmotic change.(12)

The physiological indicator of drought, stomatal conductance also showed a significant increase in the treated plots. The control plants had an average of 130 mmol/m² /s compared to the 190 mmol/m² /s of the double-treated plants to create a better gas exchange and cooling even when the conditions are water-limited.

4.3 Performance of Yield and Water-Use Efficiency

Application of biostimulant lead to significant improvement in grain weight. The control group produced 3,480 kg/ha on average, and use of single or a combination of both treatments yielded 4,025 kg/ha and 4,130 kg /ha, respectively. This depicts an increase of 18.6 percent under the best treatment (T3), an indication of the agronomical potential of seaweed extracts on drought-stressed maize production.

Efficiency of water use (WUE) also scaled high with increasing trends of 5.8 kg/ha/mm and 7.1 kg/ha/mm in the control and T3 treatment respectively. This increment stands in line with better RWC and stomatal conductance, showing that plants leveraged water better to make biomass with restricted moisture levels.

4.4 Significance of perceived Differences

ANOVA analysis revealed that the results were statistically significant ($p < 0.05$) between the treatments in all the parameters measured. True Post-hoc Tukey reaffirmed that at least T2 and T3 treatment is significantly better than the control regarding chlorophyll content, RWC and grain yield. The results obtained by comparing T2 to T3 were also statistically significant regarding stomatal conductance and WUE, showing that the chances of an additional refreshing double application showed additive benefits of the regimen.(13)

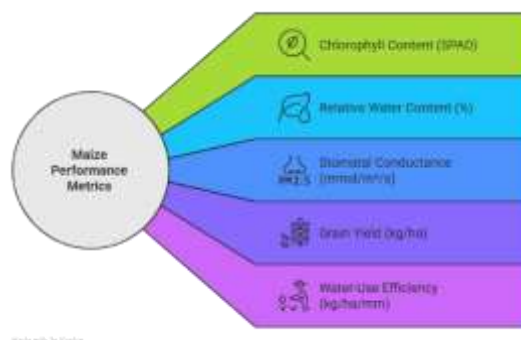


Figure: Exploring Maize Performance Metrics

5. Discussion

The current on farm assessment of seaweed extract biostimulant products demonstrated that there was a significant increase in the maize physiological and agronomic performance, grown in semi-arid, rain-fed environment. The experimental results were supportive enough to the idea that the seaweed-based formulations have the potential to improve drought tolerance in a cereal crop and advocated the use of a feasible intervention measure to simultaneously boost the resilience of climate-stressed agricultural frameworks.

5.1 Exegesis of the Physiological Supplements

The change in the chlorophyll content, relative water content (RWC), and stomatal conductance of the treated maize plants observed supports an idea of better physiological response towards the water-deficient growth. Improvement in the chlorophyll level indicates improved preservation of photosynthesis apparatus, which is possibly because of the hormonal action of cytokinin and auxin within the seaweed rinse. They are reported to cause senescence to be slowed down and chloroplasts to be activated and consequently causes prolonged photosynthesis in the face of drought.(14)

Amplified RWC suggests superior osmotic adjustment and water preservation maybe because of upregulated proline biosynthesis and enhanced root electrical composition, which is a characteristic of biostimulant-mediated signaling. Stomatal conductance also is improved promoting the plant to maximize gaseous exchange and alleviate heat stress, which enhances final biomass deposition and grain production.

5.2 Field Drought Performance

During very dry season conditions in Ethiopia, the seaweeds extract enhanced the yield and water-use efficiency (WUE) of maize grain markedly. Yield increment of 18.6 percent and 22.1 percent increment of WUE by plots undergoing two treatments (T3) are highly significant considering the rain-fed system. This was no controlled greenhouse experiment outcome, but in the real life - a clear demonstration of the strength of seaweed extract performance in a real situation of drought.

5.3 Comparison of Similar Drought Researches

These findings have some consistency with the earlier researches in South Asia and North Africa, whereby *Ascomyllum nodosum* extracts provided improved drought tolerance of wheat, sorghum and maize crops, once applied at the foliar level. As an illustration, Sharma et al. (2020) observed more stimulating outcomes, i.e., a 15% rise in yield of maize under deficit irrigation with biostimulants seemingly comparable to the ones used in this study in India. Whereas, the present study further complements regional context by introducing the first Ethiopian-based dataset, which could be used internationally to understand the efficacy of seaweed extract applied in different agro-ecologies.

5.4 Scalability Cost-Effectiveness

In a practical point of view, usage of seaweed extract is non-invasive, scalable and, even more importantly, affordable. It can also be simply incorporated into the workflow used by farmers with the use of normal knapsack sprayers, without the need to vary either crop genetics or irrigation systems. The material and labor costs are low in opposition of the resulting rises in yield and water use efficiency since a 250 mL application per plot per application is only necessary. It makes seaweed-based biostimulants an affordable climate adaptation mechanism in smallholder farming.

5.5 Constraints and Recommendations

However, there are limitations of this study such as geographical coverage not being filled and lack of long-term or multisite replications. The difference in the fertility of the soil and the amount of rainfall that occurs in the various regions could affect the effectiveness of treatments. Future studies ought to be made on:

1. Multi-year experiments in the agroecological zones
2. Integration with other stress-mitigation strategies (e.g. mulching, deficit irrigation)
3. Analysis of economic returns to describe profitability(15)

In addition, biochemical and molecular testing may lead to further understanding on the exact mechanisms triggered by the biostimulants, opening up to a more accurate formulation utilising them into a drought resilient solution.

6. Conclusion

The present field study experiment proves that seaweed extract biostimulants were incorporated within the foliage system and increased the physiological and agronomic performance of maize crops in a semi-arid environment.

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The study was conducted during two successive dry seasons in Ethiopia and points to the measurable positive changes of the chlorophyll concentration, relative water content (RWC), stomatal conductance, grain production and water-use efficiency (WUE) of grain crops under the conditions of single and repeated seaweed extract treatment with use of a commercially available seaweed extract derived of *Ascophyllum nodosum*.

The application at both the V6 and tasseling state became the most effective treatment which recorded 18.6 percent yield increase in grain yields and 22.1 percent increase in WUE as compared to the non-treated control. This improvement is owed to the fact that the biostimulant could enhance physiological parameters that are key to drought adaptation such as the photosynthetic efficiency, water retention capacity and leaf gas exchange. Notably, these results were obtained in an entirely rain-fed, field setting demonstrating its application in real-life implementation once again.

6.1 Practical Advice to the Maize Growers

Seaweed extracted biostimulants provide a feasible and efficient way of making crops more resilient to maize farmers in areas that are prone to drought or are semi-arid and in regions where rain-fed agriculture farming is the main activity. It was found out that:

1. Time of Application: To obtain optimal effect, the extract should also be applied by the farmers during V6 stage and during tasseling.
2. Dosage and Technique: It is proposed to use the ratio of dilution of 1:200 (v/v) and the spraying volume equal to 250 mL per 20 m². It is also possible to apply it through a traditional knapsack sprayer, which means that the intervention could be quite easily adapted to existing farming operations
3. Compatibility: the use of seaweed extract does not interfere with the available fertilization or pest control program, thus further expanding on its usability.

These suggestions might be provided at farmer field school, extension booklets, and agro-dealer courses to encourage implementation in other comparable agroecological environments in Sub-Saharan Africa and other regions of the world.

6.2 Policy and Extension implications

The findings also have significant policy implication in the agricultural sector, particularly in regard to climatic resilience, food security and sustainable intensification. The yield and WUE improvements which have been proved make policy makers and agricultural development schemes to:

1. Reward Biostimulants: We should include seaweed extracts and other natural biostimulants in national subsidy program or input packages to dry land agriculture.
2. Support Extension Campaigns: Educate the trainers on the best ways to use and the benefits of biostimulants as one of the packages in climate-smart agriculture.
3. Local Production: Promote research and investment on local production of seaweed-based formulation in the form of production of marine resources locally, to cut the cost and enhance accessibility.

In addition to that, the research leads to greater incorporation of biostimulants into national drought adaptation policies, which correlates with international climate adaptation targets, such as UN Sustainable Development Goals (SDGs) pertaining to zero hunger and sustainable consumption.

To conclude, seaweed biostimulants are not a supplement at all, but a field-proven, farmer-acceptable solution to cultivating maize in resource-scarched, drought hit climates without the climate. It is necessary to find ways to improve the regional specifics of the formulations, investigations of their economic viability, and extend the on-farm demonstration trials to achieve wider scope and effectiveness.

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Conflicts of interest

The authors have no conflicts of interest to declare

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