Original Research Article

Combined analysis over years on the influence of foliar application of *Kappaphycus*seaweed based biostimulant on the yield of two different varieties of black gram (*Vigna mungo* (L.) Hepper)

Abstract

Experimentswere conducted in a split plot design with the different concentrations of *Kappaphycusalvarezii* seaweed extract (KSWE;0%, 5%, 10%, 15%, and 20%) along with recommended dose of fertilizers (RDF) assigned to the main plots and varieties (TAU-1 and DBGV-5) assigned to the sub-plots at the net house facility of CSIR-Central Salt & Marine Chemicals Research Institute during the Kharif season of 2019 and 2020,in order to investigate the influence of (KSWE on two black gram varieties, DBGV-5 and TAU-1, at a single location over two years. KSWE was foliarly applied 3 times at 20, 40 and 60 DAS and various yield and yield attributes were measured. Combined analysis over the years revealed that the response of black gram varieties to KSWE varied between years, with significant improvements in yield and its attributes in 2020 as compared to 2019. KSWE concentration at 5% concentration was optimal, enhancing seed yield by 14.1% over the control and improving most yield parameters except pod length and seeds per pod. No significant interaction was observed between KSWE concentration and variety, indicating consistent effectiveness across different black gram varieties.

Introduction

Biostimulants derived from seaweeds have now-a -days gained prominence as a means for improving crop growth and productivity around the globe in a sustainable way (Shukla et al. 2019). The beneficial effects of these seaweed based biostimulants range from enhancement of growth characteristics such as plant height, number of branches, improvement in physiological parameters such as leaf area, crop growth rate, net assimilation rate, improvement in photosynthesis, increasing flowering, enhanced root growth and nutrient uptake, favorably altering the soil microbial dynamics as well as resistance to pest and diseases (; Trivedi et al. 2023). Biostimulants are derived from a wide variety of seaweeds prominent among them being *Ascophyllum nodosum*, *Ecklonia maxima*, *Sargassum*spp, *Laminaria* spp. etc.,.Recently biostimulant obtained from the seaweed *Kappaphycusalvarezii*(KSWE) has also gained impetus with several commercial products being developed (Trivedi et al. 2023). Further,the beneficial effects of the use of KSWE on various crops such as maize (Gandhi et al. 2024), sugarcane (Singh et al. 2018; Karthikeyan and Shanmugan 2017), rice (Sharma et al. 2017), and pulses such as green gram (Pramanick et al.2013) and soybean (Rathore et al. 2009) have been reported. Further, there are reports on the improvement of crop growth and yield following application of seaweed extracts on black gram (Dwivedi et al. 2014; Pramanick et al. 2016; Ammitte et al.2021). However, there are no studies that report the effect of seaweed based biostimulants (seaweed extracts) on the performance of the crop over the years. In addition, there are no reports that evaluate the effects of foliar application of KSWE on two different varieties of black gramDBGV-5 and TAU-1 at a single location. Thus, in the present study the influence of KSWE on yield and yield attributes on two different varieties of black gram, namely, DBGV-5 and TAU-1 at a single location over two years was tested and the pooled data is presented.

Material and Methods

The experiments were conducted at the net house facility of CSIR-Central Salt & Marine Chemicals Research Institute in Bhavnagar, Gujarat (21° 44’ 57.6” N; 72° 08’ 39.3” E) during the Kharif season (July-September) of 2019 and 2020. A split-plot design was employed, with the different concentrations of KSWE (0%, 5%, 10%, 15%, and 20%) along with recommended dose of fertilizers (RDF) assigned to the main plots and varieties (TAU-1 and DBGV-5) assigned to the sub-plots. Of the total ten treatments, each treatment was replicated three times, with each replication comprising at least ten pots, not including the boundary pots. The pots were filled with mixture consisting of black soil, red soil and farmyard manure in the ratio of 6:3:1. The soil mixture was characterized using standard protocols.In addition, NPK was applied in the ratio of 25:50:25 uniformly to all the pots through urea, single super phosphate and muriate of potash. The mean maximum temperature in 2019 and 2020 was 32.45 and 32.55°C, respectively, while the mean minimum temperature was 25.25 and 22.0 °C respectively.The humidity (%), precipitation (mm) and solar radiation (W m^{-2}) during 2019 was 83.78, 9.26 and 201.17 while it was 75.92, 3.86 and 205.23, respectively, in 2020. The crop was predominantly rain fed however, each the pot was irrigated with 500 mL of water as and when required during non-rainy periods (of more than 3 non rainy days). The mean pH of the soil was 7.45, while the mean organic carbon content, available N, P and K were 1.7%, 198 kg ha^{-1}, 253 kg ha^{-1}, and 1922 kg ha^{-1}, respectively. Foliar application of KSWE was carried out 3 times at 20 (vegetative stage), 40 (flowering stage) and 60 (pod filling stage) daysafter sowing (DAS) during the years 2019 and 2020. The spray volume was 5 mL per plant at 20 DAS and 13 mL during the subsequent sprays.The control plants were sprayed with water containing preservatives. The method of preparation of KSWE has been earlier described (Trivedi et al, 2018). For controlling hoppers and pod borer imidacloprid and dichlorvos were sprayed twice at 0.2 and 0.3% respectively during both the years. Hand weeding was done as when required. The crop was sown on 5th July and harvested on 28th September during 2019 while it was sown on 22nd August and harvested on 9th December during 2020. The mature pods were collected at harvest and various yield and yield attributes such as pod length, number of pods per plant, pod weight per plant, number of seeds per pod, shell weight, total number of seeds per plant, seed yield per plant from at least 6 plants in each replication during both the years. Data analysis was carried out using MSTATC software (Michigan, USA) and Microsoft excel using a two factor randomized complete block design with split plot combined over years.Pooling of data and analysis of variance was carried out only for those parameters whose variance was found to be homogenous following a F test. The test weight did not satisfy the assumption and hence was not included in the analysis.

Results and Discussion

The response of the two black gram varieties to the foliar application of *K.alvarezii* seaweed based biostimulant was significantly different in both the years (Table 1 and 2). The parameters number of pods per plant, pod weight per plant, shell weight and total seeds per plant were higher by 60.2%, 61%, 65.4% and 42.6% in 2020 over 2019. However, pod length and number of seeds per pod were higher in 2019. Increase in pod length and total number of seeds per pod led to increase in pod weight and eventually the seed yield per plant increased significantly by 51.2% in the year 2020 over 2019. Among the two main factors that were tested and combined over years, the pooled analysis of variance revealed that the main effect KSWE concentration was significant for all the parameters that were tested, except for pod length and number of seeds per pod. Among the different concentrations of KSWE that were tested, foliar application at 5% concentration was found to be better in improvingmost of the yield and yield attributes, except pod length and seeds per pod,not only with respect to control but alsowith respect to other KSWE concentrations that were tested. The seed yield was enhanced by 14.1% over control at 5% (Table 1 and 2). The increase in seed and pod weight over control as well as other KSWE treatments was primarily on account of increase in the number of pods per plant and total seeds per plant in 5% KSWE treatment. The increase in pod number and total seeds in 5% KSWE treatments was 9 and 10.7%, respectively, over control. Further a significant interaction effect was observed between KSWE concentration over the years with respect to the parameter pod length wherein 5%

KSWE application increased the pod length over the respective control. In addition, the pod length of 2019 was higher than that which was observed in 2020. However, at 15% KSWE the pod length increased with respect to control while decreasing in the year 2020 as compared to pod length of 2019.The pooled data further revealed that there was no effect of the second main factor, namely, variety on yield and its attributes (Table 2). The interaction of the variety over the years revealed that the response of all the other yield and yield attributes, except pod length and seeds per pod was similar in both the varieties that were tested over the years (Table 2). The response of the variety TAU-1 was similar in 2019 and 2020 with respect to the pod length. However, contrasting observations were made in DBGV-5 variety wherein the pod length was significantly lower in 2020 as compared to that measured in 2019 (Table 1). Further, the seeds per pod was significantly higher in 2019 as compared to 2020 in both the TAU-1 and DBGV-5 varieties. There was no significant interaction effect between the factors KSWE concentration and varieties over the years for the any of the yield or yield attribute parameters that were tested (Table 1 and 2).

Foliar application of *Kappaphycus* seaweed based biostimulant on the two different black gram varieties revealed that only the factor KSWE and that too when applied at 5% concentration was able enhance seed yield per plant. Increase in KSWE concentration above 5% concentration did not bring about any significant yield enhancement with respect to control showing that the optimum or threshold level of KSWE concentration to elicit response in blackgram under the given conditions was 5% independent of the varieties that were tested. Similar response to KSWE foliar application has also been observed in sugarcane where in 5% concentration was found to be optimum (Singh et al. 2018). Similarly, Ammitte et al. (2021) have reported enhancement in the yield of black gram variety Shekar-2 (KU300), following foliar application (twice at 20 and 40 DAS) of KSWE at a concentration of 5% along with RDF. However, in one of the field experiments conducted to evaluate the efficacy of KSWE on TAU-1 black gram variety following two foliar applications at 40 and 52 DAS, improvement in grain yield was observed when KSWE was applied at concentrations greater than 5% with the highest yield being observed at 10% KSWE (Jadhao et al. (2015). In addition, Dwivedi et al (2014) and Pramanick et al. (2016) have shown improvement in yield and other parameters of black gram following two foliar applications of KSWE at all the tested concentrations. These results clearly show that the optimum dose varies in different agroclimatic conditions which may be due to the inherent genetic make of the varieties that were tested, the meteorological conditions and edaphic factors. Further, the optimum dosage may also depend on the frequency of application as well as the time of application as empirically proved in the case of maize by Trivedi et al. (2017). Significant differences in yield and yield attributes obtained during both the years in the present study might be on account of the variation in the meterological parameters especially temperature, humidity and solar radiation as well as changes in the soil nutrient parameters of the potting mix.In the present study, application of KSWE did not elicit any significant changes in the yield response between the two varieties indicating that KSWE may function independent of the varieties in improving crop response. However, this needs to be verified by taking many varieties released in a particular agro-climatic zone and testing out their efficacy.

Conclusion

Analysis of the pooled data revealed that the optimum dose of *Kappaphycus* seaweed based biostimulant to elicit response under the given conditions was 5% concentration, the lowest tested concentration beyond no further improvement in yield was observed in black gram. The response to KSWE application was independent of the varieties that were tested. However, further experiments are need to empirically prove this hypothesis.

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Table 1: Pooled yield parameters in black gram varieties as affected by application of different concentrations of Kappaphycus seaweed extract (KSWE). Values represented are means of pooled data of two years 2019 and 2020. Means represented by different superscripted alphabets in a column are significantly different at $p < 0.05$ according to Least Significant Difference(LSD).

KSWE concentration (%)	Variety	Year	Pods plant^{-1} (g)	Pod length (number)	Seeds pod^{-1} (g)	Shell weight (g)	Total seeds plant^{-1}	Seed yield plant^{-1} (g)	Pod weight plant^{-1} (g)
		2019	55.783^{b}	4.713^{a}	6.273^{a}	5.890^{b}	350..367^{b}	15.071^{b}	20.932^{b}
		2020	89.347^{a}	4.503^{b}	5.585^{b}	9.740^{a}	499.533^{a}	22.785^{a}	33.692^{a}
0			70.845^{b}	4.585	5.895	7.520^{b}	413.667^{b}	18.112^{b}	26.082^{b}
5			77.237^{a}	4.650	5.997	8.566^{a}	458.083^{a}	20.669^{a}	29.852^{a}
10			69.814^{b}	4.607	5.916	7.421^{b}	405.667^{b}	18.071^{b}	26.091^{b}
15			72.654^{b}	4.593	5.887	7.793^{b}	421.583^{b}	18.819^{b}	27.185^{b}
20			72.274^{b}	4.606	5.951	7.702^{b}	425.750^{b}	18.939^{b}	27.350^{b}
	TAU-1		70.807	4.620	5.931	7.678	417.500	18.566	26.814
	DBGV-5		74.323	4.596	5.928	7.922	432.400	19.290	27.810
0		2019	52.052	4.641^{b}	6.153	5.319	321.833	13.643	18.962
		2020	89.639	4.530^{c}	5.638	9.720	505.500	22.581	33.201
5		2019	60.208	4.757^{a}	6.355	6.347	383.667	16.747	23.094
		2020	94.267	4.543^{c}	5.640	10.785	532.500	24.651	36.611
10		2019	54.561	4.707^{ab}	6.262	5.645	337.000	14.617	20.262
		2020	85.067	4.507^{cd}	5.571	9.196	474.333	21.526	31.921
15		2019	56.096	4.753^{a}	6.307	6.115	355.333	15.525	21.641
		2020	89.211	4.434^{d}	5.466	9.471	487.833	22.112	32.729
20		2019	55.997	4.709^{ab}	6.289	5.875	354.000	14.825	20.700
		2020	88.550	4.503^{cd}	5.612	9.530	497.500	23.053	33.999
0	TAU-1		66.821	4.567	5.806	7.100	387.167	16.912	24.465

	DBGV-5		74.870	4.604	5.986	7.939	440.167	19.312	27.698
5	TAU-1		75.731	4.665	6.029	8.594	455.000	20.680	29.755
	DBGV-5		78.743	4.634	5.966	8.538	461.167	20.718	29.950
10	TAU-1		68.242	4.627	5.925	7.310	401.333	17.547	25.536
	DBGV-5		71.385	4.586	5.907	7.532	410.000	18.595	26.647
15	TAU-1		71.772	4.613	5.898	7.770	418.500	18.631	26.932
	DBGV-5		73.535	4.573	5.875	7.886	424.667	19.007	27.437
20	TAU-1		71.467	4.628	5.995	7.688	425.500	19.063	27.380
	DBGV-5		73.080	4.584	5.906	7.716	426.000	18.815	27.319
	TAU-1	2019	54.740	4.632[b]	6.148[b]	5.711	338.467	14.563	20.274
		2020	86.873	4.608[b]	5.713[c]	9.646	496.533	22.570	33.354
	DBGV-5	2019	56.825	4.794[a]	6.399[a]	6.010	362.267	15.580	21.590
		2020	91.820	4.399[c]	5.458[d]	9.835	502.533	22.999	34.031
0	TAU-1	2019	49.597	4.514	5.874	4.858	291.667	12.170	17.028
		2020	84.044	4.620	5.738	9.342	482.667	21.653	31.902
	DBGV-5	2019	54.506	4.768	6.433	5.781	352.000	15.116	20.896
		2020	95.233	4.440	5.539	10.097	528.333	23.509	34.500
5	TAU-1	2019	60.685	4.655	6.264	6.474	383.667	17.067	23.541
		2020	90.778	4.675	5.795	10.713	526.333	24.292	35.968
	DBGV-5	2019	59.730	4.858	6.447	6.220	383.667	16.426	22.646
		2020	97.756	4.410	5.484	10.857	538.667	25.009	37.253
10	TAU-1	2019	51.651	4.652	6.164	5.341	319.000	13.506	18.847
		2020	84.833	4.601	5.686	9.278	483.667	21.588	32.224
	DBGV-5	2019	57.470	4.761	6.360	5.949	355.000	15.727	21.676
		2020	85.300	4.412	5.455	9.115	465.000	21.463	31.618
15	TAU-1	2019	55.267	4.668	6.170	5.915	342.333	14.914	20.830
		2020	88.278	4.559	5.626	9.485	494.667	22.348	33.035
	DBGV-5	2019	56.926	4.837	6.444	6.315	368.333	16.136	22.451

		2020	90.144	4.309	5.306	9.458	481.000	21.877	32.423
20	TAU-1	2019	56.500	4.672	6.269	5.966	355.667	15.156	21.122
		2020	86.433	4.583	5.721	9.411	495.333	22.970	33.693
	DBGV-5	2019	55.494	4.747	6.310	5.784	352.333	14.495	20.278
		2020	90.667	4.422	5.503	9.649	499.667	23.136	34.359

Table 3: Mean square, calculated F value, p value and its significance in two black gram varieties treated with different concentrations of KSWE and combined over years.

	Pod length (g)	Pods plant^{-1} (g)	Pod weight plant^{-1} (g)	Seeds pod^{-1} (g)	Shell weight (g)	Total seeds plant^{-1}	Seed yield plant^{-1} (g)
Mean square	0.662	16898.099	2442.453	7.098	225.825	333598.8	892.419
F(Y)	51.620	74.143	63.387	196.407	53.272	33.443	45.734
p-value	0.0020	0.0010	0.0013	0.0002	0.0019	0.0044	0.0025
Significance	**	***	**	***	**	**	**
Mean square	0.007	97.347	28.422	0.025	2.456	4830.9	13.643
F(K)	1.464	5.703	8.708	1.040	9.204	7.296	6.988
p-value	0.2594	0.0048	0.0006	0.4174	0.0005	0.0015	0.0019
Significance	ns	**	***	ns	***	**	**
CV (K)	1.543	5.693	6.615	2.592	6.623	6.055	7.382
Mean square	0.008	185.437	14.900	0.000	0.894	3338.2	7.842
F(V)	0.907	2.928	1.691	0.002	1.250	1.767	1.624
p-value	0.3523	0.1025	0.2083	0.9613	0.2768	0.1987	0.2171
Significance	ns	ns	ns	ns	ns	ns	ns
CV (V)	2.087	10.966	10.869	3.210	10.842	10.227	11.609
Mean square	0.016	20.254	5.292	0.041	0.761	1231.9	2.788
F(K × Y)	3.234	1.187	1.621	1.720	2.853	1.860	1.428
p-value	0.0400	0.3542	0.2174	0.1947	0.0584	0.1667	0.2701
Significance	*	ns	ns	ns	ns	ns	ns
Mean square	0.515	30.701	1.531	0.961	0.045	1178.1	1.298

F(V × Y)	55.702	0.485	0.174	26.533	0.063	0.624	0.269
p-value	0.0000	0.4943	0.6813	0.0000	0.8046	0.4389	0.6098
Significance	***	ns	ns	***	ns	ns	ns
Mean square	0.004	20.726	5.265	0.034	0.370	1371.5	3.339
F(K × V)	0.380	0.327	0.597	0.939	0.517	0.726	0.692
p-value	0.8202	0.8563	0.6687	0.4618	0.7239	0.5844	0.6064
Significance	ns	ns	ns	ns	ns	ns	ns
Mean square	0.007	22.045	4.415	0.026	0.202	639.0	1.941
F(K × V × Y)	0.792	0.348	0.501	0.718	0.283	0.338	0.402
p-value	0.5441	0.8422	0.7353	0.5894	0.8856	0.8489	0.8049
Significance	ns	ns	ns	ns	ns	ns	ns

F tab at 1% & 5% for KSWE are 4.73 and 3.01 respectively for d.f= 4,16; similarly for F(variety) tab values are 8.096 and 4.351 for 1% & 5% respectively at d.f=1,20;; ns- not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$;