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Validation of POPs for Bt Cotton Production in Highly *Calcareous* Soils

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A B S T R A C T

Package of practices for highly *Calcareous* soils were developed, tested and validated to supply optimum NPKSZn and B content in index leaf of Bt hybrid cotton during boll formation stage, such as seed treatment (S.Tr) with NPK *consortia* soil having *Azotobacter sp*. strain MTCC-3853 + *Rhizobium leguminoserum*- strain MTCC-99 + PSB: *Bacillus megatherium var*. *phosphaticum* strain MTCC-24121, MTCC 2412, *Bacillus licheniformis* strain-MTCC-2312, *Bacillus subtilis* strain MTCC-736 + KMB: K mobilising bacteria *Acido thiobacillus ferroxidans* strain: 5370; *Pseudomonas fluorescens migula*: strain 2659 to encourage soil biological activities in low OC soil along with 75% RDF only. Bentonite sulphur 20 kg ha^{-1} soil application (SA) + RDF; *Sagarika* S. Tr along with twice foliar sprays (FS) at squaring and flowering + RDF; Nano ZnO 4% S. Tr. twice FS + RDF during 2019, exception was during 2020, the magnitude was 50% less due twice torrential and 5 times medium rains during July and August months upset the physiology of cotton, shedded all the fruiting bodies, unable to maintain index leaf NPK, besides heavy pink bollworm attack. All these treatments were applied with 100% RDF granular, split, spot application having 6.5%Sulphur containing, N: P_2O_5: K_2O @ 22.5 kg ha^{-1} twice on 15, 30 DAS and twice as Urea at 45 and 60 DAS, Bentonite Sulphur 20 kg ha^{-1} or seed treatment and foliar applications of bio-stimulants *Sagarika* @ 0.02% and twice foliar applications of the same @ 0.002% and Nano ZnO @ 0.004% twice produced 67-86 ppm Zn in index leaf of the Bt hybrid cotton, similar to that of chelated Zn 0.5%. This POPs produced 161-287 kg lint ha^{-1} i. e. more than double 38 to 50% due to better nutrient uptake, biomass production, boll number with more than INRs. 16 to 30, 000/- ha^{-1} profitability and 1.59 to 2.15 C:B ratio, FUE 1.6 to 1.2 and lowest cost of production in both the years. Large scale OFTs in Kalmeshwar Tq, Nagpur district (M.S.) India in highly *Calcareous* soils, confirmed the beneficial effect of soil application of Bentonite sulphur or sulphur containing complex fertilizers @ RDF with and without two winter irrigations in late September besides seed treatment with PGPRs or Bio-stimulants before sowing and twice foliar applications at squaring and flowering stages with $ZnSO_4$ 0.5% or nano ZnO 4% 0.004% or Bio-stimulants 28% 0.002% along with Urea 2% and Boron 0.3% or cheleted micronutrients 0.5% along with WSF 17:44:0 and 0:0:50 2.0% in doubling the seed cotton yields besides reducing pre mature leaf reddening by maintaining optimum index leaf nutrient content with 40 bolls $plant^{-1}$.

Introduction

Soil fertility and fertilizer management

Calcareous soils were formed in semi arid to humid tropics under different *litho* and *pedogenic* process covering 70% of the total geographical area of India (Pasricha *et al.,* 2001; Raju *et al.,* 2018 ab; Shingare *et al.,* 2022). *Calcareous* soils have high free calcium, permeable, sloppy and erodible with embedded limestone hard pan can restrict choice of crops, root growth, duration and often demands frequent light irrigations (Raju *et al.,* 2012, 2012; Raju, 2017; Raju and Deshmukh, 2018; Shingare *et al.,* 2022). *Calcareous* soils were low in organic carbon, N, Mg, S, Fe, Cu, Mn and Zn and B but medium in available P, rich in K (Table4) expressing visible symptoms after the onset of reproductive stage under soil moisture deficit interacting with free lime interferes with the uptake and availability of nutrients (Shingare *et al.,* 2022; Rajuand Soniya, Thakare, 2014). Major fraction of applied N has been lost through volatilization, P transformed into calcium P, however, available before cotton flowering Cotton significantly responded in 17% in low to medium P (5 to15 mg kg^{-1}) soils due to availability of band applied granular phosphorous upto 50 days as conformed by tracer studies (Rochester and Till, 2007). *Calcareous* soils require a proper balanced split, spot, multiple nutrient application of granular N, P, K, S, Zn, B coated, simple, mixed or complex fertilizers has been recommended to reduce volatilization and fixation losses on clay, organic matter and $CaCO_3$. Loss of urea as $NH4^+$ in runoff, NO_3 movement were traced with P^{32}, P^{33} studies in *Vertisols* with light, medium and heavy rains as NO_3 moved 15, 60 and >100 cm away from the placement (Patra and Thomas, 1997; Rochester and Till, 2007). Soil PK nutrients balance were negative @ 25-35 kg ha^{-1} soil application and positive @50-75 kg ha^{-1} with response upto 60 kg ha^{-1} (More and Agale, 1991). Three times KNO_3 foliar application during reproductive stage in cotton + pigeon pea strip cropping cropping systems on in *calcareous Vertisols* produced 15% higher lint yield (Mullins and Burmester, 1990). PGPRs solubilize P, K, Zn, fixes nitrogen, produce phyto-hormones like kinetin, GA_3, IAA, ACC-deaminase and siderophores, hydrogen cyanide, and ammonia, which enhances crop growth, yield, and fertility status of the soil (Sheng, 2005; Dudhade and Gadakh, 2021; Rani *et al.,* 2022). *Pseudomonas, Bacillus, Acinetobacter, Gluconacetobacter, Thiobacillus* and *Rhizobium* are some of the most powerful Zn solubilizing strains that can be efficient to boost soluble Zn in the soil which will benefit plant growth and yield (Rajinder and Sukhminderjit, 2020). Bacterial strain-PS-4 solubilized 253 ppm of ZnO and produced a high quantity of lactic acid 169 g ml^{-1} and acetic acid 471 g ml^{-1}. Further, *in vitro* studies demonstrated higher production of auxin, gibberellic acid and siderophore by PS-4 (Rani *et al.,* 2022) and proven field performance in cotton-wheat system at ICAR, CICR, Regional Station, Sirsa, Haryana, India by Raju and Uma 2008. *Consortia* of compatible Zn-SB, P-SB, K-SB/ P-MB, *Pseudomonas*, *Trichoderma* and *Bacillus* strains as potential inoculants cum seedling protectants (Ahmed *et al.,* 2021). Highest rate of Zn release was by *Pseudomonas fluorescence* strain Ur-22 36 mg L^{-1} which was associated with decrease in pH 6.8 to 4.2 (Hashemnezad *et al.,* 2021). Soil application of *Bacillus megatherium* broth showed significantly highest Zn solubilization followed by *Trichoderma viride* and *Pseudomonas striata* (Pawar and Sayed, 2012). *Bacillus sp* inoculum produced significantly higher seed cotton yield 120 kg ha^{-1} which was equal to 45 kg ha^{-1} P_2O_5 fertilizer applied on clay loam soil with pH 8.3, and available P 10 mg kg^{-1}. Seed treatment with *Azotobactor*, PSB and KMB *Consortium* + 100 % RDF recorded higher *Sorghum* grain yield of 2137 kg ha^{-1}, net returns of Rs.55,422 ha^{-1} with B: C ratio of 3.05 (Egamberdiyeva *et al.,* 2004; Sheng, 2005; Dudhade and Gadhak, 2021). Significant interaction was recorded with Zinc, Sulphur 500 $kgha^{-1}$ along with *Thiobacillus inoculam* was equal to that of Sulphur 1000 kg ha^{-1}. Phosphorus content was significantly increased in cotton plants inoculated with *Rhizobium meliloti* combined with *PSB* with no inoculation has resulted very low P uptake in plants. *Bacillus* KF974682 was

found to solubilize the maximum amount of phosphorous 0.13 g/ml and *Bacillus edaphicus* NBT increased by 26% K content (Sheng, 2005). Benonite sulphur and seed treatment with P, S, Zn solubilizers and K mobilsers are also useful to make nutrients available to cotton (Egamberdiyeva *et al.*, 2004; Sheng, 2005; Raju, 2017; Raju and Deshmukh, 2018; Raju *et al.*, 2018). Crop-stimulants for better uptake, foliar correction of deficit nutrients in regular, chelated and nano forms are also being tested (Raju and Deshmukh, 2018 and Raju, 2023) in station trials at ICAR, Central Institute for Cotton Research, Nagpur and on farm trials in *calcareous Vertisols* with Bt hybrid cotton + pigeon pea strip cropping system in *Kalmeshwar*, north Nagpur.

Changing rainfall pattern

Calcareous soils are thirsty, it was felt only in 2019 under 25 days delayed onset of monsoon with dry sowing of Bt hybrid cotton without fertilizer application with weak germinated seedlings under desiccating atmospheric conditions under 42% less rainfall in 37% less rainy days received towards the end of the June month in both the years (Table 1, Fig. 1. 2).

However, July, 2019 had a seedling drought in first three weeks followed by total last wet rainy week received 16% extra rains in 11% less rainy days (Table 2). July, 2020 month had received two heaviest (60-100 mm) rainy days on 14.7.20 and 23.7.20, remaining light to medium rains (25-50 mm), 89% rain in prolonged 13% extra rainy days over normal effective rainfall. Similarly, August, 2019 month had also received 22% extra rains in 67% more rainy days, where two heavy rainfall events temporarily sub-merged cotton ridges, four medium rainfall events filling cotton furrows and six light rainfall events and the same number were effective contributing to the soil moisture. August, 2020 month received 50% annual rainfall out of 60% effective rain events 30% were light, 13% were medium 3% or two events were heavy rains in the beginning and end of the month received 70% of the monthly or equal to that of normal monthly rainfall i.e. 200 mm or 83% extra rains in double number of rainy days being wettest month in both years interfering intercultural operations, weeding and top dressing of fertilizer applications over the normal. Similarly, September, 2019 was also wettest month received eight light and two medium rainfall events with 63% extra rains in very prolonged 2.25 times more number of rainy days, while in September, 2020 month received four light and three medium rainfall events with 10% extra rains in 88% more number of rainy days being wettest month in both years delaying the normal intercultural operations, weeding and top dressing of fertilizer applications. October, 2019 month had received two light rainfall events with 16% less rainfall 1.5 times more number of rainy days, while in October, 2020 month had received three light one medium rainfall events with 51% extra rains in 2.5 times more number of rainy days being wettest month in both years.

Materials and Methods

A field experiment was conducted with Bt hybrid cotton *Rashi*-659 with ten nutrient management treatments and 4 replications in RBD layout in highly *Calcareous* soils with root limiting calcium carbonate layer at 30 to 45 cm below the soil (Table 3, 4). Experimental trial was for two years during 2019, 2020 monsoon season at ICAR, Central Institute for Cotton Research, Nagpur, Research Farm (21.15, 79.1). *Calcareous* soil depth and calcium carbonate content and seasons were diverse, therefore, they were not pooled together, but discussed their impact with reference bench mark independently.

Nutrient management treatments were Tr. 1. Control No recommended dose of fertilizers i.e. 90:45:45 kg ha^{-1} N: P_2O_5: K_2O (RDF), which serves as indicator for early warning of nutrient deficiencies in the absence of external fertilizer application besides estimating the seasonal changes in natural soil fertility and calculating the fertilizer application economics and use efficiency in the highly *Calcareous* soils. Tr.2. Seed treatment (S.Tr) with

NPK *consortia* having *Azotobacter sp*. strain MTCC-3853 + *Rhizobium leguminoserum*- strain MTCC-99+ PSB: *Bacillus megatherium var. phosphaticum* strain MTCC-24121, MTCC 2412, *Bacillus licheniformis* strain -MTCC-2312, *Bacillus subtilis* strain MTCC-736 + KMB: K mobilising bacteria *Acido thiobacillus ferroxidans* strain: 5370; *Pseudomonas fluorescens migula*: strain 2659 along with 75% RDF only. Tr. 3. 100% RDF through nitro phosphate *Suphala* 15:15:15 having 6.5% Sulphur complex fertilizer as basal dose followed by twice urea top dressing at 45 and 60 DAS, which was compared with the present general recommendation of balanced fertilization to hybrid cotton. Tr. 4. 100% RDF + $ZnSO_4$ 20 kg ha^{-1} yr^{-3} + elemental sulphur (100%) 20 kg ha^{-1} yr^{-3} + Borax 5 kg ha^{-1} yr^{-3} as soil application (SA) at the time of sowing i.e.10 days before the basal dose of fertilizer application. Tr. 5. RDF + S.Tr. Zn solublizer (ZnSB) *Acido thiobacillus ferroxidans* : strain 5370 *Pseudomonas fluorescens migula* : strain 2659. Tr.6. Zn SO_4 20 kg ha^{-1} yr^{-3} as SA for deficit soils as soil application 10 days before after complex fertilizer application to avoid Zn fixation. Tr. 7. Tr. 3 + Borax 5 kg ha^{-1} yr^{-3} SA for deficit soils. Tr. 8. RDF + Bentonite Sulphur (80% Sulphur and 15-20% Na) 20 kg ha^{-1} yr^{-3} as SA for medium to deficit soils. Tr. 9. RDF + *Sagarika* seed treatment 0.02% of seed weight. Tr.10. Tr. RDF + *Sagarika* seed treatment and twice foliar sprays (FS) 0.002% at squaring and flowering stage. Tr. 11. RDF + *Sagarika* granules 25 kg $ha^{-1}yr^{-1}$ as SA. Tr.12 RDF + nano ZnO 4% twice foliar sprays (FS) 0.004% at squaring and flowering stage. Tr. 13. RDF 75% N: P_2O_5: K_2O only. Tr. 14. RDF 75% + Zn SO_4 20 kg ha^{-1} yr^{-3} as SA for deficit soils + chelated commercial formulation of micronutrients foliar sprays (FS) 0.005% twice at squaring and flowering stage. Tr. 14. RDF 75% +@RD of Zn B Fe SA + twice chelated micronutrients foliar sprays Tr. 15. RDF 75% +Nano seed treatment twice FS of the same Tr. 16. RDF 75% +Seed treatment with NPK *consortium* and Zn solubiliser.

Soil of the experimental site was analyzed as per the standard protocol, before the start of the experiment. Field seedling growth observations on plant height, primary root length, shoot length, number of laterals, root and shoot biomass (Table 3) were recorded, analyzed and interpreted with weather conditions at one month from the date of sowing in both the years.

Most recently matured index leaf top 4^{th} leaf, samples were collected at 115, 122, 134, 170 during 2019 and 30, 60, 77, 115 DAS in 2020, twice washed in dilute HCl, followed by tap water and twice with distilled water. Leaf samples were shade dried, powdered and digested by wet acid digestion method (Matusiewicz, 2003). Soil nitrogen (N) was analyzed by micro kjeldahl method and index leaf N was analyzed calorimetrically by modified Nessler's reagent method (Plaza *et al.,* 2013). Soil organic carbon (SOC) was analyzed by Walkley and Black's (WBC) reverse titration method (Jha *et al.,* 2014). Soil Boron was estimated by HWE Azomethionine - H method (Sarkar *et al.,* 2014). Soil and plant potash was analyzed by flame photometer (Bares *et al.,* 1945). Soil and plant phosphorous by Olsen's method Ascorbic acid blue, Vanebdo molybdate yellow colour method respectively (Alcala *et al.,* 2014). Mg, Zn, Fe, Cu, Mn DTPA extracted soil samples and wet acid digested plant samples were analyzed by AAS as per AOAC procedures (Uddin *et al.,* 2016). *Calcareous* soil was found to be deficient in all parameters except medium in available P and rich in available K (Table 4).

Results and Discussion

Impact on seedling growth

Plant height, primary root length, shoot length, number of laterals, root and shoot biomass were significantly influenced at 30 DAS in only July,2019 by the seed treatment with NPK *consortia* along with 75% RDF and with *Sagarika* seed treatment along with 100% RDF, but not in 2020 due to 50% excess rains. (Table 3). Bt hybrid cotton seedling performance at 30 DAS in July, 2019, was significantly influenced only when it received 119 mm rain in 18 rainy days (RD) its performance was upset at the end of month another 150 mm 54%

received only in two continuous days on 31.6.19 and 1.7.19. Plant height and root length, were non significant due to shortage of soil moisture, compared to July, 2020, when cotton was planted after receiving 100 mm pre monsoon rains in 11 days before the sowing of Bt hybrid cotton. After sowing 102 mm rains was received in 17 rainy days followed by 177 mm i.e. 47% rain only in two rainy days (14 July and 23rd July). All bio- stimulants as seed treatment in highly *calcareous* soils were known to have the bio stimulating effect due to its Zn, cytokinin and other humic and fulvic acids content. *Calcareous* soils are known to be thirsty followed by hidden hunger, when it reaches peak demand at early reproductive stage of any crop grown on it. These experiences were in agreement with those observed by Pasricha *et al.*, (2001) and Shingare *et al.*, (2022).

Table.1 Rainfall mm and number of rainy days during 2019, 2020 seasons.

	Rainfall mm			**Rainy days (RD), Effective RD**				
Months	**2019**	**2020**	**Normal**	**2019**	**ERD**	**2020**	**ERD**	**Normal**
June	132	126	208	7	5	8	6	11
July	398	305	481	13	6	17	16	20
August	343	**515**	314	20	13	**24**	17	12
September	**299**	201	228	**22**	**18**	15	**8**	12
October	48	86	27	3		5	5	3
Total	**1172**	**1147**	1231	**62**	**42**	**64**	**52**	58

Table.2 Soil analysis data of Experimental site 2019 season.

S. No.	**Soil content**	*Calcareous* soils		Typical *Vertisols*	
		Content	Category	Content	Category
1	pH	7.66	Normal	7.68	Normal
2	EC mS/m	2.58	Normal	2.41	Normal
3	Organic Carbon %	0.39	Low	0.10	Low
4	Available Nitrogen kg ha^{-1}	180	Low	180	Low
5	Available P_2O_5 kg ha^{-1}	17	Medium	19	Medium
6	Available K_2O kg ha^{-1}	580	High	550	High
7	Available Zinc ppm	0.55	Low	0.65	Low
8	Available Mg ppm	0.22	Low	0.24	High
9	Available B ppm	0.25	Low	0.50	Medium
10	Available $CaCO_3$%	29.6	High	22.3	High

Table.3 Impact of Bio stimulants as seed treatment on Bt hybrid cotton seedling performance.

Tr.No.	Treatments	Plant height cm		Primary root length cm		Lateral roots numbers		Root biomass g plant		Shoot biomass g plant	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
T1	Control	13.1	22.2	26.3	11.8	11.0	4.8	1.56	1.2	18.0	4.8
T2	RDF 75%+ Seed Tr NPK *consortia*	14.9	28.5	**29.8**	13.8	10.3	7.2	1.46	2.1	**22.0**	7.2
T3	RDF 90:45:45N:P_2O_5:K_2O	**13.4**	24.0	26.8	10.0	4.5	4.7	2.12	2.0	**17.8**	4.7
T4	RDF +SA Zn 20 S20 B5 kg $ha^{-1}yr^{-3}$	12.0	24.7	24.0	12.3	4.5	5.0	2.06	2.6	20.3	5.0
T5	RDF **+Zn solubiliser** Seed Tr	13.0	25.3	26.0	11.8	7.5	7.7	2.35	1.9	17.6	7.7
T6	RDF +SA Zn 20 kg $ha^{-1}yr^{-3}$	11.8	25.9	22.5	13.5	11.0	7.8	1.69	2.1	21.5	7.8
T7	RDF +SA Borax 5 kg $ha^{-1}yr^{-3}$	**12.3**	23.8	24.5	12.5	9.0	8.2	1.60	1.7	**17.5**	8.2
T8	RDF +B Sulphur 20 kg $ha^{-1}yr^{-3}$	**15.1**	26.0	30.3	11.5	9.0	5.7	1.83	2.0	**21.9**	5.7
T9	RDF ***+Sagarika* Str.**	**14.6**	30.0	29.3	**13.3**	9.5	**8.5**	**2.07**	**1.5**	**22.8**	**8.5**
T10	**9+*Sagarika*FS**	**16.5**	24.0	34.5	**10.8**	9.3	**6.0**	**1.55**	**1.9**	**21.8**	**6.0**
T11	RDF +*Sagarika* Gr 25 kg ha^{-1}	13.1	27.0	26.3	11.3	13.0	6.5	1.41	2.2	**23.4**	6.5
T12	RDF +Zn nano fertilizers 2 FS	**12.0**	27.3	24.0	13.5	13.3	7.2	2.56	2.1	**12.3**	7.2
T13	RDF 75% N:P_2O_5:K_2O		27.3	25.8	13.5	12.0	8.2	1.58	1.8		8.2
T14	RDF75%+ SA Zn20 B5 Fe 20 kg $ha^{-1}yr^{-3}$ + chelated FS		25.5	25.0	12.0	10.5	6.5	1.73	1.9		6.5
T15	RDF 75% +**Nano seed treatment FS**		26.5		**13.3**		**4.5**	**2.05**	**1.8**		**4.5**
T16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .		25.5		12.0		6.0	1.18	6.0		6.0
	S.E± C.D. 5 %	S	1.60	S	1.08	S	0.97	0.24	0.97	S	0.97
	CD at 5 %	0.6	NS	9.48	NS	4.91	NS	NS	NS	1.6	NS

Table.4 POPs agronomic performance in Bt hybrid cotton 2019.

S.No.	Treatments	Boll number plant^{-1}	Yield g plant^{-1}	Bio mass Tonne ha^{-1}	Lint Yield kg ha^{-1}	C:B ratio	Net returns Rs. 000 ha^{-1}	Cost Rs kg^{-1} seed cotton	FUE kg^{-1} lint kg^{-1} fertilizer
1	Control	27	48	4.97	254	1.1	2.5	73	
2	Seed Tr (S Tr) with NPK *consortia* + RDF 75%	31	67	**6.68**	324	1.6	18.9	50	0.52
3	N:P_2O_5:K_2O RDF 90:45:45 kg ha^{-1}	36	53	5.72	415	1.9	31.6	41	0.89
4	RDF + Zn20 S20 Borax 5 kg ha^{-1} Soil application (SA)	36	57	6.37	454	1.9	35.2	41	1.11
5	RDF + S. Tr with Zn Solublizer	36	53	6.01	522	2.2	46.7	35	1.49
6	RDF +Zn 20 kg ha^{-1} SA	38	61	4.77	363	1.7	23.3	47	0.61
7	RDF + Borax 5 kg ha^{-1} SA	38	**75**	5.53	515	2.2	45.2	36	1.45
8	RDF + Bentonite S 20 kg ha^{-1} SA	31	65	6.00	541	2.3	48.9	35	1.59
9	RDF +*Sagarika* S Tr 0.2%	34	73	6.57	514	2.2	45.5	36	1.44
10	9+*Sagarika* 2 foliar sprays (FS)	35	63	5.27	539	2.2	48.4	35	1.58
11	RDF +*Sagarika*granules SA 25 kgha^{-1}	35	52	5.76	418	1.8	31.0	42	0.91
12	RDF + Nano Zn 0.04% 2 FS	39	65	5.54	538	2.2	48.3	35	1.58
13	RDF75%	27	62	5.06	389	1.9	29.8	41	0.75
14	RDF 75% + Zn 20 SA + Chelated FS	34	**72**	6.04	449	2.0	35.7	40	1.08
	S.E± C.D. at 5 %	2.4	1.04	**0.90**	0.35				
	Sig	S	S	NS	S				
	SED /CD+5 %	11	11	0.20	85				

Table.5 POPs agronomic performance in Bt hybrid cotton 2020

Tr.No.	Treatments	Lint yield Kg ha^{-1}	FUE Kg kg^{-1}	Cost of production kg^{-1}	Net returns Rs ha^{-1}	C:B Ratio	Index leaf Zn PPM			
							30	60	90	120
T1	Control	268		35	8586	1.92	35.5	35.2	88	89
T2	RDF 75%+ NPK solubiliser	325	0.42	39	9116	1.72	30.8	41.5	104	56
T3	RDF NPK only	321	0.29	42	7997	1.59	30.2	33.6	84	67
T4	RDF +Zn20 S20 B5 kg **ha^{-1}** soil	293	0.14	49	5144	1.36	31.6	19.7	49	73
T5	RDF +Zn solubiliser	**446**	0.99	33	**15116**	2.02	32.9	40.2	100	57
T6	RDF +Zn20 **ha^{-1}** soil app	258	-0.06	54	3269	1.23	30.6	18.3	46	66
T7	RDF +Borax **ha^{-1}** soil app	372	0.58	39	10509	1.73	33.4	17.8	44	83
T8	RDF +Bentonite Sulphur20**ha^{-1}**	256	-0.06	52	3907	1.29	27.9	16.9	42	38
T9	RDF + *Sagarika* streatment	**486**	1.21	31	**17374**	**2.15**	33.0	20.3	51	43
T10	RDF + *Sagarika* streatment+FS	**477**	1.16	34	**15871**	1.99	35.1	19.1	48	84
T11	RDF +*Sagarika* granules	410	0.79	38	12089	1.79	31.2	18.6	46	39
T12	RDF + nano Zn FS	394	0.70	39	10991	1.71	30.3	17.3	43	39
T13	RDF75%	317	0.36	40	8691	1.69	30.9	16.5	41	82
T14	RDF 75% +Zn B Fe SA + chelated foliar sprays	346	0.58	43	8139	1.54	33.5	18.3	46	36
T15	RDF 75% +**Nano seed treatment FS**	**447**	0.99	33	**15155**	**2.03**	31.1	16.9	42	32
T16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .	331	0.35	45	7433	1.50	31.9	16.8	42	60
	C.D. at 5 %	125			82	0.82	2.4	2.3	6.7	30

Table.6 Bt cotton index leaf Nitrogen content shallow *Vertisols* with *calcalcareous* sub strata.

S.No.	Treatments	2019				2020			
		Days after sowing							
		115	122	134	170	30	60	77	115
1	Control	3.1	5.3	3.0	3.2	4.45	2.25	2.66	1.32
2	RDF 75%+ NPK solubiliser	4.3	5.6	3.0	2.7	4.83	2.36	2.35	1.47
3	RDF NPK only	4.1	5.0	3.0	3.4	4.52	2.72	2.96	1.32
4	RDF +Zn20 S20 B5 kg **ha^{-1}** soil	3.9	4.2	3.8	3.3	3.04	2.66	2.65	1.31
5	RDF +Zn solubiliser	3.2	5.0	3.2	2.9	4.91	2.26	2.73	1.44
6	RDF +Zn20 **ha^{-1}** soil app	3.2	4.3	3.3	3.3	4.35	2.48	2.77	1.40
7	RDF +Borax **ha^{-1}** soil app	3.4	4.9	3.4	3.1	3.53	2.41	2.16	1.60
8	RDF +Bentonite Sulphur20**ha^{-1}**	3.1	5.4	2.9	2.8	4.47	2.09	2.63	1.33
9	RDF + *Sagarika*streatment	3.7	4.2	2.8	3.3	3.82	2.09	2.62	1.32
10	RDF + *Sagarika*streatment+FS	3.5	5.4	2.8	3.2	3.72	2.71	2.86	1.30
11	RDF +*Sagarika* granules	3.2	4.6	2.5	3.0	5.41	2.96	2.83	1.38
12	RDF + nano Zn FS	3.9	4.8	3.9	2.9	4.27	2.55	2.62	1.41
13	RDF75%	3.1	4.9	2.5	2.7	4.34	2.40	3.11	1.44
14	RDF 75% +Zn B Fe SA + chelated foliar sprays	3.7	4.4	3.9	3.3	3.81	2.79	2.67	1.47
T15	RDF 75% +**Nano seed treatment FS**					4.43	3.21	2.70	1.34
T16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .					4.05	2.80	2.67	1.33
	C D±5%	0.6	0.5	0.4	0.2	0.48	0.44		0.10
	Sig	NS	NS	NS	NS	NS	NS	0.36	NS

Table.7 Bt cotton index leaf Phosphorous content shallow *Vertisols* with *calcalcareous* sub strata.

S.No.	Treatments	2019				2020			
		Days after sowing							
		115	122	134	170	30	60	77	115
1	Control	0.51	0.33	0.29	0.50	**0.12**	**0.21**	**0.43**	**0.58**
2	RDF 75%+ NPK solubiliser	0.60	0.42	0.33	0.45	**0.12**	**0.21**	**0.34**	**0.52**
3	RDF NPK only	**0.87**	**0.42**	**0.31**	**0.46**	**0.13**	**0.14**	**0.51**	**0.65**
4	RDF +Zn20 S20 B5 kg **ha^{-1}** soil	0.55	0.44	0.26	0.47	**0.11**	**0.20**	**0.43**	**0.65**
5	RDF +Zn solubiliser	0.66	0.43	0.26	0.42	**0.13**	**0.18**	**0.39**	**0.69**
6	RDF +Zn20 **ha^{-1}** soil app	0.81	0.39	0.31	0.64	**0.14**	**0.21**	**0.39**	**0.65**
7	RDF +Borax **ha^{-1}** soil app	0.58	0.36	0.28	0.48	**0.14**	**0.22**	**0.27**	**0.51**
8	RDF +Bentonite Sulphur20**ha^{-1}**	0.52	0.37	0.30	0.45	**0.15**	**0.17**	**0.36**	**0.55**
9	RDF + *Sagarika*streatment	0.70	0.47	0.29	0.86	**0.15**	**0.17**	**0.34**	**0.71**
10	RDF + *Sagarika*streatment+FS	0.84	0.45	0.29	0.62	**0.11**	**0.20**	**0.38**	**0.65**
11	RDF +*Sagarika* granules	0.65	0.37	0.28	0.48	**0.12**	**0.20**	**0.47**	**0.56**
12	RDF + nano Zn FS	0.56	0.35	0.31	0.52	**0.10**	**0.17**	**0.43**	**0.68**
13	RDF75%	0.55	0.40	0.27	0.52	**0.11**	**0.21**	**0.52**	**0.73**
14	RDF 75% +Zn B Fe SA + chelated foliar sprays	0.65	0.40	0.28	0.45	**0.11**	**0.21**	**0.39**	**0.62**
T15	RDF 75% +**Nano seed treatment FS**					**0.10**	**0.20**	**0.52**	**0.81**
T16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .					**0.12**	**0.23**	**0.38**	**0.65**
	CD±5%	1.6	0.5	0.7	0.7	**26.5**	21.24	**1.61**	**2.2**
	Sig					**2.6**	**2.07**		

Table.8 Bt cotton index leaf Potash content in shallow *Vertisols* with *calcalcareous* sub strata.

S.No.	Treatments	2019				2020			
		Days after sowing							
		115	122	134	170	30	60	77	115
1	Control	1.2	0.4	0.4	0.5	**2.6**	**2.07**	**1.61**	**2.2**
2	RDF 75%+ NPK solubiliser	1.6	0.6	0.5	1.1	**3.0**	**2.20**	**1.72**	**2.6**
3	RDF NPK only	1.6	0.5	0.7	0.7	**1.7**	**2.32**	**1.61**	**2.1**
4	RDF +Zn20 S20 B5 kg **ha^{-1}** soil	1.5	0.5	0.6	0.7	**2.2**	**2.11**	**1.44**	**1.2**
5	RDF +Zn solubiliser	1.5	0.7	0.6	0.6	**2.4**	**1.87**	**1.42**	**2.5**
6	RDF +Zn20 **ha^{-1}** soil app	1.5	0.6	0.6	0.6	**3.2**	**2.08**	**1.49**	**1.1**
7	RDF +Borax **ha^{-1}** soil app	1.5	0.5	0.6	0.8	**1.5**	**2.12**	**1.58**	**1.1**
8	RDF +Bentonite Sulphur20**ha^{-1}**	1.5	0.6	0.6	0.8	**3.3**	**1.80**	**1.68**	**1.1**
9	RDF + *Sagarika*streatment	1.6	0.6	0.6	0.7	**3.3**	**1.80**	**1.58**	**1.3**
10	RDF + *Sagarika*streatment+FS	1.6	0.7	0.7	0.6	**2.3**	**2.27**	**1.55**	**1.2**
11	RDF +*Sagarika* granules	1.4	0.5	0.5	0.7	**2.4**	**1.99**	**1.53**	**1.2**
12	RDF + nano Zn FS	1.5	0.6	0.6	0.7	**3.2**	**1.78**	**1.68**	**1.1**
13	RDF75%	1.2	0.4	0.5	0.7	**1.6**	**1.54**	**1.53**	**0.1**
14	RDF 75% +Zn B Fe SA + chelated foliar sprays	1.5	0.6	0.6	0.8	**2.1**	**2.13**	**1.58**	
T15	RDF 75% +**Nano seed treatment FS**	0.07	0.04	0.04	0.07	**1.8**	**2.35**	**1.58**	
T16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .	0.19	0.12	0.12	0.20	**2.3**	**2.52**	**1.59**	
	CD±5%	1.2	0.4	0.4	0.5	**0.37**	0.25	**1.61**	
	Sig	1.6	0.6	0.5	1.1	**1.08**	NS	**1.72**	**0.4**

Table.9 Bt cotton index leaf Zinc content in shallow *Vertisols* with *calcalcareous* sub strata.

S.No.	Treatments	2019				2020				
		Days after sowing								
		115	122	134	170	30	60	77	115	135
1	Control	48	37	28	37	35.5	35.2		35.5	35.2
2	RDF 75%+ NPK solubiliser	61	87	48	53	30.8	41.5		30.8	41.5
3	RDF NPK only	50	63	47	44	30.2	33.6		30.2	33.6
4	RDF +Zn20 S20 B5 kg **ha**$^{-1}$ soil	65	84	38	45	31.6	19.7		31.6	19.7
5	RDF +Zn solubiliser	67	90	50	45	32.9	40.2		32.9	40.2
6	RDF +Zn20 **ha**$^{-1}$ soil app	65	88	41	45	30.6	18.3		30.6	18.3
7	RDF +Borax **ha**$^{-1}$ soil app	54	58	48	56	33.4	17.8		33.4	17.8
8	RDF +Bentonite Sulphur20**ha**$^{-1}$	54	63	40	55	27.9	16.9		27.9	16.9
9	RDF + *Sagarika*streatment	64	66	51	53	33.0	20.3		33.0	20.3
10	RDF + *Sagarika*streatment+FS	65	64	51	56	35.1	19.1		35.1	19.1
11	RDF +*Sagarika* granules	67	55	40	53	31.2	18.6		31.2	18.6
12	RDF + nano Zn FS	67	83	66	53	30.3	17.3		30.3	17.3
13	RDF75%	49	37	37	51	30.9	16.5		30.9	16.5
14	RDF 75% +Zn B Fe SA + chelated foliar sprays	86	85	66	54	33.5	18.3		33.5	18.3
15	RDF 75% +**Nano seed treatment FS**	11	14	16	9	31.1	16.9		31.1	16.9
16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .	12	14	25	13	31.9	16.8		31.9	16.8
	C D±5%	4	5	6	3	2.4	2.3		NS	6.7

Table.10 Bt cotton index leaf Iron content in shallow *Vertisols* with *Calcareous* sub strata.

S.No.	Treatments	2019				2020		
		Days after sowing						
		115	122	134	170	30	60	77
1	Control	208	198	155	237	71.7	88	89
2	RDF 75%+ NPK solubiliser	201	196	139	554	76.7	104	56
3	RDF NPK only	193	211	141	454	76.5	84	67
4	RDF +Zn20 S20 B5 kg **ha**$^{-1}$ soil	188	135	165	558	66.0	49	73
5	RDF +Zn solubiliser	267	281	153	557	73.0	100.4	57
6	RDF +Zn20 **ha**$^{-1}$ soil app	205	167	188	587	87.9	46	66
7	RDF +Borax **ha**$^{-1}$ soil app	253	161	144	640	86.9	44	83
8	RDF +Bentonite Sulphur20**ha**$^{-1}$	169	204	148	445	85.2	42	38
9	RDF + *Sagarika*streatment	241	204	159	421	80.9	51	43
10	RDF + *Sagarika*streatment+FS	254	139	144	513	78.7	48	84
11	RDF +*Sagarika* granules	179	170	140	521	81.7	46	39
12	RDF + nano Zn FS	199	180	181	562	85.4	43	39
13	RDF75%	188	176	189	545	79.2	41	82
14	RDF 75% +Zn B Fe SA + chelated foliar sprays	193	188	203	442	93.8	46	36
15	RDF 75% +**Nano seed treatment FS**	16	24	22	33	69.7	42	32
16	RDF 75% + **seed treatment** NPK consortium and Zn solubiliser .	46	68	NS	96	81.5	42	60
	Sig	16	25	27	14	14	16.8	10.42

Yield and yield attributes

Package of practices tested for highly *Calcareous* soils with significant agro economic performance were T5: RDF 75%+ *Consortia* of Zinc solubilising and K mobilising bacteria (ZnSB, KMB) as seed treatment (S.Tr) ;T8: RDF+ Bentonite sulphur 20 kg ha^{-1} SA, T9: RDF+ *Sagarika* S.Tr, T10: RDF+ *Sagarika* S.Tr along with twice foliar sprays (FS) at squaring and flowering, T12: RDF + Nano ZnO S. Tr. twice FS during 2019 except T9 during 2020, the magnitude was 50% less in 2020 (Table 5) due to torrential rains (Table 2) upset the physiology of cotton, shedded all the fruiting bodies, unable to maintain index leaf NPK, besides heavy pink bollworm attack during the year.

All these treatments were applied with two split applications of *Suphala* a sulphur containing, granular, 100% RDF @22.5 kg ha^{-1} N: P_2O_5: K_2O twice on 15, 30 DAS (RCF 15:15:15 Nitro phosphate) and twice as *Neem* coated Urea @ 22.5 kg N ha^{-1} at 45 and 60 DAS i.e. squaring and flowering stage of cotton when NPK demand is highest. This along with Bentonite sulphur 20 kg ha^{-1} (T8) or seed treatment and foliar applications of bio-stimulant *Sagarika* @ 0.02% and foliar applications of the same @ 0.002% (T10) or nano ZnO @ 0.004% (T12) twice at squaring and flowering stage produced 67-86 ppm Zn in index leaf of the Bt hybrid cotton (Table 4, 5, 7), similar to that of chelated Zn twice foliar applications.

This produced 287 kg lint ha^{-1} in 2019 and 125 to 161 kg ha^{-1} during 2020 i. e. more than double to 38 to 50% due to better N, P, K, Zn nutrient uptake, biomass production, boll number in highly *Calcareous* soils with more than Rs. 16 to 30, 000/- ha^{-1} in profitability and 1.59 to 2.15 C:B ratio, FUE 1.6 to 1.2 and lowest cost of production in both the years (Table 4, 5). The results for Bentonite sulphur as soil amendment in highly *Calcareous* soils to solubilise and improve the supply of P and Zn were in agreement with those observed by Nayak and Patil (2012); Sisodiya *et al.*, (2016); Raju *et al.*, (2018, 2023) in improving the agronomical performance of crops. The results for seed treatment with N fixing, P and Zn solubilising PGPRs were in agreement with those observed by Raju *et al.*, (2008) in *Vertisols*, by Raju *et al.*, (2018) in highly *Calcareous* soils, by Uma and Raju (2008) in red soils. The results for *Sagarika* seed treatment and foliar application of the same for cotton as crop growth stimulant by Raju *et al.*, (2018, 2023). The results in highly *Calcareous* soils for foliar correction of nutrients deficiencies by spraying twice at squaring and flowering with nano ZnO 0.004% or $ZnSO_4$ 0.5% and Boron 0.3% along with WSF NPK to correct nutrient deficiencies were in agreement with those observed by Raju *et al.*, (2018, 2023).

Index leaf nutrient content

The year 2019 and 2020 both had 40% excess rains over the water requirement of cotton, same rainfall and rainy days, in 2020 each two monthly heavy rainfall events in July and August, 2020 (Table 1, Fig, 1, 2) followed by four medium rainfall events in both the years received major amount of rains during rainy season caused run off and leaching of all the applied water soluble nutrients N, P, K, and Zn.

Fertilizer nitrogen (N) was applied in two splits as complex nitro phosphates and three splits of as *neem* coated prilled urea. Year 2019 had a seedling drought of 25 days, followed by two torrential and four medium rains also leads to N, P, K, Zn, B runoff and leaching losses as confirmed by Patra and Thomas, (1997) for leaching losses Raju (2023) for these index leaf nutrients status in similar soils. Index leaf NPK could be maintained near optimum only in 2019 (Table 6), which was not even 50% of normal index leaf NP due to runoff and leaching losses (Table 6), which was due to delayed fertilizer urea application followed by 24 and 15 rainy days in August and September, 2020 months respectively, resulted in 50% less bolls and lower lint yields (Table 4, 5). Similar, to nitrogen, phosphorous was also could not be maintained after 45 DAS during 2020, which were far below, the

threshold levels. This is a big challenge for cotton agronomist since 2020-2023, to apply and deliver NP fertilizer in index leaf during excess rains or delayed monsoon, which is changing the growth and reproductive physiology of cotton. The results in highly *Calcareous* soils for foliar correction of nutrients deficiencies by spraying twice at squaring and flowering with nano ZnO 0.004% or $ZnSO_4$ 0.5% and Boron 0.3% along with WSF NPK to correct nutrient deficiencies were in agreement with those observed by Raju *et al.*, (2018, 2023).

The conclusion is package performance in highly *Calcareous* soils was split application of 100% RDF granular, split, spot application of two basal 3 top dressing of fertilizer nutrients three days after the heavy rain during the crop growth (15-60 days) are required along with Bentonite sulphur 20 kg ha^{-1} or Sulphur containing complex fertilizer. Seed treatment with PGPRs or bio-stimulant to a dry sown cotton is a must. Foliar application of bio-stimulant *Sagarika* @ 0.002% or nano ZnO @ 0.004% or Zinc sulphate 0.5% twice at squaring and flowering stage produced 87-67 ppm Zn in index leaf, similar to chelated Zn twice foliar applications produced 170-287 kg lint ha^{-1} i.e. more than double due to better N, P, K, Zn nutrient uptake, biomass production, boll number in highly *Calcareous* soils with more than Rs. 16-30, 000/- ha^{-1} profitability. The year with higher rain fall like 2020 dry bed planting of cotton followed by using a sticker spraying 3 times (45-75 days) with WSF along with insecticides, fungicides, Urea 2%+Zinc sulphate 0.5% or WSF 17:44:0 + 0:0:50 2.0% along with chelated micronutrients 0.5% or nano ZnO 0.004% and Boron 0.3% are also required to augment the soil supply.

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