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Potential of the entomopathogenic fungus *Beauveria bassiana* as an endophyte in cotton

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Abstract
Eight isolates of *Beauveria bassiana* were applied to cotton plant by four inoculation methods *viz*., seed immersion, seed coating, soil drenching and foliar application to establish *B. bassiana* as endophyte in cotton. One month later, the cotton plant leaves, stems and roots are sampled to evaluate endophytic fungal colonisation. Samples are cut into multiple sections, surface sterilized and incubated in media. The media is inspected every 2-3 days to observe fungal growth associated with plant sections and occurrence of *B. bassiana* recorded to estimate the extent of its endophytic colonisation. All the isolates were able to colonise the cotton plant. Among the four methods, plants inoculated by foliar application method recorded highest colonisation followed by soil drenching with no negative effect on plant growth or survival. *B. bassiana* isolate 8 was the best coloniser followed by *B. bassiana* 1 and *B. bassiana* 3. The result of colonization of *B. bassiana* as endophyte was revealed that, *B. bassiana* can able to establish as endophyte in cotton plants, causing no harmful effects and might provide an alternative method for biological control.

Keywords: Cotton, *Beauveria bassiana*, endophytes, colonisation, microbial control

1. Introduction
The indiscriminate use of pesticides and chemical fertilizers in agriculture has raised a number of ecological problems such as resistance development in plant pathogens and pests, environmental pollution and negative impacts on human health. In this context, beneficial microorganisms are now integrated as part of integrated pest management practices [1], which allow a significant reduction in the use of synthetic chemicals. The entomopathogenic fungi *Beauveria bassiana* is natural enemy of a wide range of insects [2] and have been extensively studied for biological control [3, 4].
Fungal entomopathogens can be used in multiple ways to protect crops against attacks of insect pests. In the last decade, several studies have shown that many entomopathogenic fungi also possess the potential to colonise plant tissues and thus to grow as an endophyte inside different plant species [5, 6]. Moreover, *B. bassiana* has been successfully established as an endophyte *via* an artificial application in a variety of crop plant species including maize, potato, cocoa, coffee, banana, date palm and sorghum [7]. Accordingly, endophytic establishment and entomopathogenic activity of *B. bassiana* in a given crop plant represents an alternative strategy for the application of this fungus in insect pest management programmes and has thus a high potential for development of new and sustainable crop protection strategies.
Enhanced endophytic expression of entomopathogen within the plant system is expected to be more advantageous than external application of bio-agents because of continuous presence and assured expression of the entomopathogens throughout the crop cycle and get protected from biotic and abiotic factors.

2. Materials and Methods
2.1 *B. bassiana* isolates inoculum preparation
Eight *B. bassiana* isolates (*B. bassiana* 1 to *B. bassiana* 8) were used in this experiment. The isolates *B. bassiana* 1 to *B. bassiana* 7 were isolated from Coffee berry borer, *Hamilton hampsei* infested with *B. bassiana* from different village of Dindigul district, Tamil Nadu, India and *B. bassiana* 8 was isolated from cotton leaf form Coimbatore. Isolated *B. bassiana* isolates were subcultured on Sabouraud dextrose agar medium supplemented with yeast extract (SDAY) (10g peptone, 20g dextrose, 5g yeast extract and 15g agar^{-1} distilled water)

and containing antibiotics (0.1g penicillin, 0.2g streptomycin and 0.05g chlorotetracycline^{-1} SDAY) in 55 mm diameter Petri dishes. The Petri dishes containing the *B. bassiana* were incubated for three weeks in the laboratory (22–30ºC, RH 65-70% and a photoperiod of 12:12 hrs). After three weeks from these cultures, *B. bassiana* suspension was prepared. The conidial concentration for each treatment were adjusted to 1 x10^8 conidia ml^{-1}. Germination test of conidia was done before inoculation of the cotton plants.

2.2 Inoculation methods

The pot culture experiments were conducted at the ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India during the period of 2015-16. To determine the potential of *B. bassiana* colonisation on cotton plants, *B. bassiana* was inoculated by four different methods: (1) Seed immersion (2) Seed coating (3) Foliar spray and (4) Soil drenching. The experiment was organized as a Completely Randomised Design with inoculum dose at 10^8 conidia/ml. For each treatment, three replications were maintained and five plants per replication were used for experiment. The experiment was conducted a total of three times.

Suraj cotton variety seeds were used for these experiments. Seed coating was done by adding 1g of *B. bassiana* conidial suspension at 10^8 concentration along with talc. Control seeds were coated with talc and deionsed water. For seed immersion inoculation, 50g of cotton seeds were immersed in 10 ml of a *B. bassiana* conidial suspension for 6 h. They were then dried on sterile tissue paper in a sterile laminar flow cabinet, sown in 15 cm dia plastic pots filled with a sterile soil and maintained in room temperature and a photoperiod of 12-12h L-D. Control seeds were immersed in a conidia-free solution of 0.01% Tween 80. For foliar spray, a hand sprayer was used to spray each seedling with 10 ml conidial suspension. Control plants were sprayed with a conidia-free solution of 0.01% Tween 80. For the soil drenching method, 10 ml conidial suspension of 10^8 concentration was applied around the root zone of each seedling. In the control, sterile 0.1% Triton X-100 applied in the same way as in each treatment mentioned above. After inoculation, each plant was covered with a plastic bag for 24 hrs to maintain a high level of humidity.

2.3 Evaluation for presence of *B. bassiana* in cotton tissues

The colonisation of *B. bassiana* was evaluated by culturing method at one month after post inoculation. The inoculated plants were kept under conditions of room temperature and natural light conditions of 12:12 h and watered daily. Stems were cut off (about 5cm above the stem base) from the roots using a sterile blade. The plant parts were rinsed thrice in sterile deionized water. The leaves were cut into 1 cm^2 sections, sterilized in a laminar airflow cabinet by dipping in 0.5% Sodium hypochlorite suspension for two minutes followed by dipping in 75% ethanol for 2 min. The tissues were dried on sterile paper towels and placed in 55 mm petri dishes containing SDAY. The medium was supplemented with antibiotics (0.1g penicillin, 0.2g streptomycin sulphate, 0.25g chloramphenicol and 0.05g chlortetracycline^{-1} SDAY) to prevent bacterial contamination. A total of 10 plants were evaluated for each treatment during the course of one time of inoculation. The petri dishes were incubated for four days at 25 ± 2 ^{0}C in the laboratory, after which all plant samples were visually examined for fungal outgrowth.

Percentage colonization was calculated as number of samples exhibiting *B. bassiana* outgrowth per total number of samples, results are expressed as the percentage of plants positive for the presence of *B. bassiana* after inoculation. The colonisation frequency data (expressed as percentages) were angular transformed. The transformed data were analysed using analysis of variance (ANOVA) performed with the software Version Infostat, 2001. For Scanning electron microscopy (SEM), young leaves inoculated with *B. bassiana* conidia were selected, cut into small pieces and then dehydrated in an ethanol series to 100% ethanol.

3. Results

3.1 Colonisation of *B. bassiana* in cotton as an endophyte

B. bassiana was able to endophytically colonise cotton plant in response to the demonstrated inoculation treatments. Placing plant parts of treated samples on medium showed emergence of *B. bassiana* by microscopic examination. *B. bassiana* was not detected in any of the control plant sections. All the inoculation techniques were successful in establishing *B. bassiana* as an endophyte, but differences observed in efficacy among the different inoculation techniques and different isolates and different plant parts.

The average percent colonization or colonization frequency varied among the endophytic *B. bassiana* isolates from 46.67% to 3.33%. The technique that resulted highest colonisation was the foliar spray, with 46.67% colonisation of leaves at thirty days post-inoculation. The highest colonization percent (46.67%) was recorded in *B. bassiana* isolate 8 followed by *B. bassiana* isolate 1 (43.33%). Irrespective of method of inoculation, among the eight *B. bassiana* isolates, the highest mean colonization percentage recorded as 19.59 in *B. bassiana* isolate 8 followed by *B. bassiana* isolate 1 as 19.17% (Table 1).

The inoculation method significantly affected the percent colonisation. Both foliar spray and soil drench methods resulted in endophytic colonization of 35% of the treated plants by *B. bassiana*. However, the extent of colonization depended on the plant part evaluated and the inoculation method used. Seed immersion method resulted with lowest mean colonisation percentage of 4%. Irrespective of inoculation methods and different isolates, leaves responded best with 16% mean colonisation and maximum of 21% colonisation by spray inoculation method. Stems also responded similarly to all inoculation methods with mean colonisation of nine percentage (Table 1).

Using Scanning Electron Microscopy on cotton seedlings inoculated with *B. bassiana*, conidial germination and hyphal growth were found to be associated with leaf. Germ tube formed from a conidium and hyphae of *B. bassiana* were observed also penetrating epithelial cells of cotton and ramifying through palisade parenchyma and mesophyll leaf tissues. Hyphae may gain access to the leaf interior through stomatal openings. The typical method of invasion is directly through the epidermal cell wall and into the leaf interior. Penetration of the epidermal cell wall shows that the plant cell wall is completely breached and the hyphae grow through the hole. Examination of hyphae inside the leaf shows that they grow through the air spaces between parenchyma cells. The fungus colonized parenchyma both intra- and intercellular (Fig. 1 & 2).

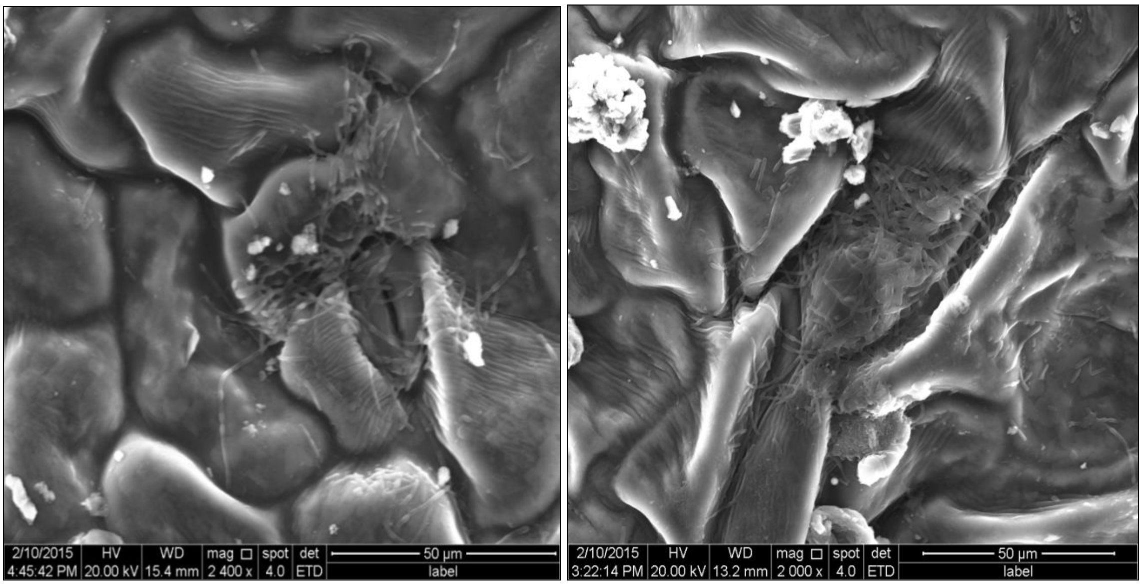

Fig 1-2: Scanning electron microscopy photograph showing *B. bassiana* colonisation on cotton leaf

Table 1: Efficacy of inoculation treatments on endophytic colonization by *Beauveria bassiana* on cotton plant (1 month after inoculation)

Isolates	Per cent colonisation										
	Seed coating		Seed immersion		Soil drenching		Foliar spray		Mean (Isolate)	Mean (Leaf)	Mean (Stem)
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem			
Beauveria bassiana 1	16.67	13.33	3.33	6.67	20.00	20.00	43.33	30.00	19.17	20.83	17.50
Beauveria bassiana 2	13.33	6.67	3.33	3.33	20.00	13.33	30.00	13.33	12.92	16.67	9.17
Beauveria bassiana 3	13.33	10.00	3.33	0.00	23.33	20.00	36.67	13.33	15.00	19.17	10.83
Beauveria bassiana 4	20.00	10.00	6.67	3.33	20.00	13.33	33.33	16.67	15.42	20.00	10.83
Beauveria bassiana 5	16.67	0.00	6.67	6.67	16.67	0.00	10.00	3.33	7.50	12.50	2.50
Beauveria bassiana 6	6.67	3.33	0.00	3.33	10.00	3.33	20.00	6.67	6.67	9.17	4.17
Beauveria bassiana 7	3.33	3.33	3.33	0.00	0.00	0.00	10.00	0.00	2.50	4.17	0.83
Beauveria bassiana 8	16.67	13.33	6.67	6.67	30.00	16.67	46.67	20.00	19.59	25.00	14.17
Mean (Method)	10.42		3.96		14.17		20.83		12.35	15.94	8.75
S.Ed	1.402	1.659	2.348	2.099	1.022	1.215	1.016	1.330			
CD (5%)	2.971	3.517	4.978	4.450	2.166	2.576	2.154	2.82			

Replications-3, Samples/replication-10

4. Discussion

In the present study, at one month post inoculation, *B. bassiana* was successfully re-isolated from the interior of stem and leaves of cotton plants, clearly indicating that cotton can serve as a suitable host for *B. bassiana* endophyte. Many factors can influence the outcome of an experiment to establish a fungal entomopathogen as an endophyte, includes crop species and the fungal entomopathogen isolate used, the concentration of the inoculum, the age of the plant during inoculations and inoculation methods.

This result indicates that foliar spray treatment with the spore suspension of *B. bassiana* is an effective method for achieving endophytic colonization of the entomopathogen in cotton. *B. bassiana* has been established as an endophyte in various plants by different methods of inoculation [8]. However, endophytic colonization of *B. bassiana*, depended upon the inoculation method, fungal isolate and plant species.

Recovery from stems and leaves also shows that *B. bassiana* can translocate throughout the plant tissues. The lack of any visual symptoms on the seedlings also would indicate that *B. bassiana* can colonize this plant without causing detriment to the host. In the current study, *B. bassiana* colonization was differed among the plant parts isolated. The reason for higher colonization on leaves than stems is not clear but could reflect differences in microbial and physiological conditions in the different plant parts. Many endophytic fungi show a certain degree of tissue specificity because they are adapted to particular conditions present in a given organ [9-11]. Differential *B. bassiana* colonization on plant parts was equally demonstrated in corn (*Zea mays* L.) and cocoa (*Theobroma cacao* L.) [12-13]. In corn, the fungus was most frequently isolated from the internode below the primary ear and less frequently from the leaf collar at the primary ear. The reason for the lack of endophytic colonisation in seeds immersed with *B. bassiana* is not clear and requires further investigation. In soil drenching method, watering of plants might have led to loss of conidia through water filtration, reducing their chances of uptake by the roots. Instead, it appears that inoculation methods tend to favour a specific pattern of local colonization. In coffee, for example, foliar sprays favour leaf colonization whereas soil drenches favour root colonization.

B. bassiana isolate 8, isolated from cotton leaf, colonized plant tissues better than other isolates. Based on the origin of the strains and the results obtained in this study, we hypothesize that *B. bassiana* isolate 8 and *B. bassiana* 1 is well adapted to endophytically colonize cotton plants. Biswas *et al*., 2013 [7] reported, the variation in colonization frequency among various *B. bassiana* strains may be due to their differential growth rate and endophytic adaptation. Ultimately, the choice of inoculation method should be guided by the intended location of the endophyte within a plant, presumably matching the niche of the target herbivore.

5. Conclusions

In conclusion, we provide evidence that important entomopathogenic fungi *B. bassiana* colonised living cotton plant and therefore may act as cotton endophytes. This can be an important ecological advantage for maintaining a natural *B. bassiana* inoculum in cotton plant. The success of artificial inoculation of *B. bassiana* as endophyte into cotton plants determines many future works, which should focus on the improvement of efficacy of endophytes against insects.

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Chapter 11

Climate Smart Agro Techniques for Yield Enhancement and Sustainability of Cotton Based System

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Introduction

Climate change will have major impact in cotton production as the crop is sensitive to moisture availability during flowering to boll development. The negative impact of climate change is reduced availability of water for irrigation. We can mitigate and adapt to climate change by adoption of scientific climate smart agro technologies in cotton based system. The potential climate smart agro technologies to enhance the yield and profitability in cotton based system during changing climate are grouped into three categories viz., water smart, energy smart and carbon smart technologies. The water smart technologies are poly ethylene mulching, bio degradable poly ethylene mulching, Drip irrigation, Poly mulch + drip irrigation, and *in situ* moisture conservation techniques to conserve and use the available moisture effectively so as to achieve more crop per drop of water. The energy smart technologies are those technologies which encourages lesser energy intensive inputs like reduction in chemical fertilizers and weedicides, Adoption of INM practices with inclusion of legumes and bio inoculants for lesser dependence on inorganic nitrogenous fertilizers and agro techniques like stale seed bed, weed smothering cover crops as tools in IWM encourages reduction in usage of herbicides to manage weeds efficiently and finally the carbon smart technologies are those involving conservation agriculture for sequestering maximum carbon in soil such as reduced tillage, crop based mulches, multi tier cropping , high density planting system etc.

Agriculture has to undergo a complete transformation to meet the twin challenges of higher productivity for the growing population and long term sustainability of productivity in the face of global climate change. Agriculture accounts for 30-40% of GHG emissions (IPCC, 2007). However, according to Cotton Incorporated (2009), cotton production could even be considered as carbon sink as the amount of carbon stored in the fiber and soil would exceed the total GHG emissions that occur while growing and ginning the crop. Cotton is popularly known as**"King of Fibres"** or **"White Gold"**. Millions of people depend on cotton cultivation, trade, transportation, ginning and processing for their livelihood. India is the only country in the world growing all the four cultivated species of cotton along with their hybrid combinations in the vast agro-climatic situations. In India, Cotton is grown in about 12 m ha with the production of 36 m.bales and 65 per cent of cotton area is under rain fed.World over, Cotton covers about 2.5% of the world's arable lands and is thus related to between 0.3% to 0.8-1.0% global GHG emissions. It is therefore not a principal source of GHG emissions. Yet, Cotton can contribute to mitigating climate change by adoption of scientific climate smart agro techniques'o meet the comfort clothing requirement of ever growing population, expanding the cotton area is not possible and enhancing the productivity is the only solution. The potential climate smart agro technologies to enhance the yield and profitability in cotton based system during changing climate are being reviewed and discussed hereunder.

1. Poly ethylene mulch technology

The practice of mulching in agriculture has been widely used as a management tool for centuries. Mulching improves the soil physical condition by enhancing aggregation and conserving soil moisture by increasing infiltration, checking losses by evaporation and run off. It also favorably modifies the soil thermal regime, retards soil erosion and improves soil health. The advantages of using plastic mulches for the production of high value vegetable crops have been recognized in the United States and the European countries. The beneficial effects of plastic mulch for enhanced water and fertilizer utilization and weed control (Fortnum, *et al.* 2000), weeds and pests (Lakshmanan, *et al.* 1995) have been reported. Higher uptake of nutrients due to higher temperature coupled with higher available soil moisture caused significant increase in root cation exchange capacity, nutrient uptake, dry matter accumulation and partitioning of assimilates under polyethylene mulching with an average yield enhancement of 1.83 to 1.90 fold in cotton and 1.95 to 2.10 fold in maize crops besides saving water (Nalayini *et al.* 2009). The water requirement of mulched cotton was 58.63 ha-cm as against conventionally grown cotton of 84.19 ha-cm thus a huge water saving due to poly mulching. Mulched cotton consumed lesser water due to lesser evaporative loss of water and weed free environment. The mulched area was free from weeds (no weedicide was used to control weeds under mulching) and almost all the weeds were effectively controlled except Cyprus spp. which could pierce through the thinner mulch of 30 micron. The effective control of weeds under poly mulching indirectly prevented the loss of water through transpiration by weeds. Higher temperature and faster mineralization under polyethylene mulching caused increased uptake of nutrients (Table 1) as evidenced from up to 2.1, 2.8 and 2.6 fold higher N,P and K uptake, respectively over conventionally planted cotton. Soil moisture conservation due to poly mulching was reported by Channabasavanna, *et al.* (1992) and Gupta and Acharya, (1993).

1.1. Thickness and colour of poly film for mulching

Various thickness poly film of 30, 50, 75 and 100 micron were evaluated and all the thickness were found suitable and were on par. Since the cost of mulch film is based on weight, the lower thickness of 30-40 micron is recommended to reduce the cost. While using thinner poly film of 30 micron, care should be taken to handle the film delicately so that the film will be used for two crops at once over laying. Differently coloured poly ethylene mulching of black , red, blue, yellow and silver colour were evaluated and it was found that irrespective of the colours, the poly ethylene mulching promoted the growth and development of cotton significantly over conventionally planted cotton. The silver colour recorded the lowest pest incidence due to reflective action. While, the yellow colour attracted the sucking pests in cotton and the yield enhancement was highest with silver colour poly film. The enhancement in seed cotton yield due to coloured poly mulching was to the tune of 1.96, 1.75, 1.71, 1.68 and 1.57 fold respectively in silver, blue, red, black and yellow colours over conventional method. We recommend dual colour poly film of silver colour top layer with black bottom layer for reflective action by silver colour and for faster mineralization by black bottom layer. Dong et al 2009 reported enhanced seed cotton yield in cotton due to adoption of intensive farming using plastic mulching in China.

Table 1: Soil temperature, Available Soil Moisture, Nutrient uptakes, rhizosphere and phyllosphere micro organisms in cotton, LRA 5166 due to poly mulching.

Characters	Poly mulched	No mulch control
Soil temperature across soil depth up to 45 cm (^{o}celsius)	29.2	27.6
Available soil moisture (%) average at 20 DAS interval	23.2	18.26
Nutrient uptake (g/plant) on 90 DAS		
Nitrogen	1.98	0.93
Phosphorus	0.25	0.09
Potassium	2.5	0.97
A.Rhizosphere soil of cotton (/gram of dry soil)		
Diazotrophs	164.2×10^{4}	63.7×10^{4}
Facultative Methylotrophs	109.2×10^{4}	26.4×10^{4}
Azospirillum	19.2×10^{4}	6.7×10^{4}
Phosphorus solubilizing bacterias	90.57×10^{3}	42.9×10^{3}
B. Phyllosphere of cotton/g of fresh leaf		
Pink Pigmented Facultative Methylotrophs	134.6×10^{4}	39.3×10^{4}
C. Root Bits (rotation maize)		
Arbuscular mycorrhizae infection (%)	90	73.3

2. Biodegradable polyethylene mulching

Polyethylene film by nature is non biodegradable, but it can be made biodegradable (oxo degradation) by addition of little quantity (4%) of prodegradant additive (d2w,a patented product from UK). The polyethylene film which was prepared with addition of prodegradant not only biodegradable after the intended use, but also utilized by soil microbes as carbon source (Michael Stephenson, 2009). Oxobiodegradable plastics sheets can be programmed at manufacturing stage to degrade soon after the harvest or until the mission is accomplished. The remaining bits in the field after harvest can be collected and buried into the soil to hasten the biodegradability. The small unnoticed leftover bits in the field may not pose environmental problem due to its biodegradable nature. Australian field trial using biodegradable mulch film on capsicum crops has shown that biodegradable plastic performs just as well a polyethylene film but also has the advantage of being able to be ploughed into the ground after harvest which can reduce the disposal costs while enriching the soil with carbon on degradation (Anon, 2004). The use of biodegradable mulch films seems to be apromising alternative because the films can degrade right in the field and the amount of waste ending up in landfills can be avoided (Kijchavengkul, *et al.* 2008).

Table 2: Seed cotton yield of ELS *Bt* cotton cv. RCHB 708 as influenced by interaction between moisture regimes and mulches

Mulches	Moisture regimes			Mean
	Drip (0.4 ETc)	Drip (0.8 ETc)	Conventional Irrigation	
No mulch (control)	3175	4070	3421	3555
Sub soil coir pith (2 kg/m^2)	4314	4067	4092	**4158**
Maize stover (5 kg/m^2)	4254	4215	3979	**4149**
Sugarcane trash (5 kg/m^2)	4208	4077	4002	**4096**
Surface coir pith (2 kg/m^2)	4111	3809	3442	**3787**
Gunny sheet mulching	4354	4250	3994	**4199**
Biodegradable polymulching	5234	4647	4679	4853
Polyethylene mulching	5641	5418	5357	5472
Mean	**4411**	4319	**4121**	**4284**
CD (p=0.05) for mulches	620			
CD (p=0.05) for moisture regimes	270.1			
CD (p=0.05) for interaction	928.5			

The study conducted by us using different crop based mulches indicated that biodegradable polyethylene performed just like polyethylene in conserving moisture, weed control and yield promotion (Table 2) under no mulch condition cotton crop responded up to 0.8 ETc while with mulches, it was sufficient to maintain the moisture regime at 0.4 ETC, thus a huge water saving due to mulches (Nalayini, *et al.* 2017).

3. Poly mulch + Drip fertigation with balanced ferilizers

Combining Drip fertigation with balanced fertilizers under poly ethylene mulching enhanced the seed cotton yield significantly with the a huge saving of irrigation water, higher yield potential with enhanced resource use efficiency. Drip + poly mulching recorded 46.1% and 86.5% higher seed cotton yield than drip irrigation without poly mulch and conventional method respectively. Balanced fertilization with 120:60:60 kgs NPK/ha along with zinc sulphate (50 kg/ha), magnesium sulphate (50 kg/ha) + Boron as Sulphur (1 kg /ha for soil application and 0.15% as foliar spraying twice during flowering to boll development stages recorded the highest (7820 kg/ha) seed cotton yield as against 3290 kg/ha recorded under conventional irrigation with NPK alone (Nalayini, *et al.* 2012).

Table 3: Seed Cotton Yield in ELS Bt cotton, RCHB 708 as influenced by Water Conservation techniques and fertilization practices

Fertilization Treatments	Water conservation Techniques			Mean
	Drip	Drip + Poly Mulch	Control	
T1 - 100% NPK	3740	5920	3290	4316
T2 - T1 + Zn SO_4	4460	6480	3720	4886
T3 - T1 + Mg SO_4	5320	6970	3820	5370
T4 – T1+ Boron	5200	7370	3910	5493
T5 – T1 + Zn SO_4+ Mg SO_4 + Boron	5760	7820	4110	5896
T6 -75 % of T5	4430	7660	3920	5336
MEAN	4820	7040	3775	
CD (P=0.05) for W	416			
CD (P=0.05) for F	599			

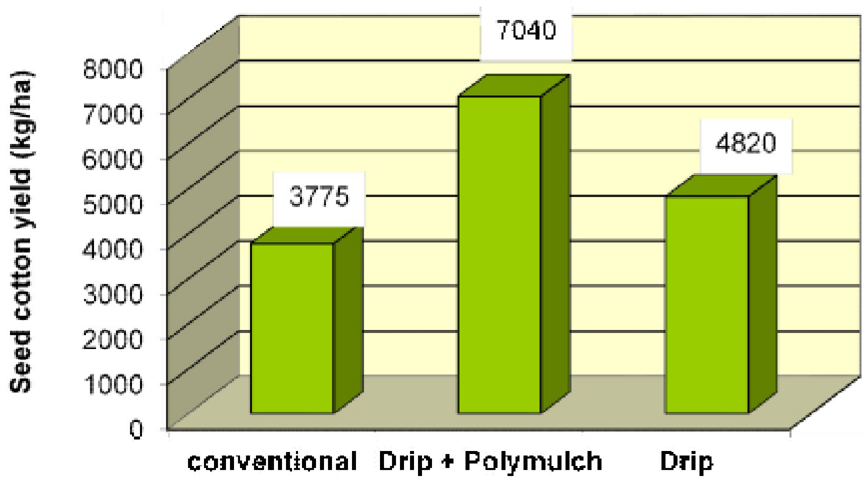

Fig. 1: Comparison of Yield potential of ELS Cotton under Drip, conventional and Drip + Poly mulching

In, drip-fertigation, where fertilizer is applied through an efficient irrigation system, nutrient use efficiency could be as high as 90 per cent. The amount of fertilizer lost through leaching could be as low as 10 per cent in fertigation whereas it is 50 per cent in the traditional one. In addition, fertilizer savings through fertigation could be to the tune of 25-50 per cent (Haynes, 1985). On medium deep clay soil at Parbhani, nitrogen application at 75 kg/ha through drip recorded comparable yield to that of 100 kg N/ha by soil application in a hybrid cotton NHH 44 (Vaishnava, *et al.* 1995). Fertilizer and water saving with high efficiency of inputs could be achieved with less cost by adopting poly tube drip system (system cost 70% less) (Sankaranarayanan, 2005).

4. Other *in - situ* moisture conservation Techniques

In situ soil moisture conservation techniques could reduce moisture stress by effectively conserving soil moisture. Preparatory tillage operation viz., ploughing and harrowing gave additional cotton yield of 100 kg ha^{-1} as compared to only harrowing before sowing at Dharward. Contour bunding is the effective soil and water management system to reduce run off and soil erosion, while increasing infiltration of rainfall (Kampen and Krantz, 1976). The other practices include graded, narrow or broad ridges or beds separated by furrows for drainage, reduce run off and soil erosion and increasing infiltration of rainfall. Forming beds (120-180 cm wide) and furrows on a grade for *insitu* water harvesting is found to be efficient in deep black soils with a rainfall of 700-850 mm (Venkateswarlu, 1980). Ridges and furrow method of water harvesting has been recorded 42 per cent increase in cotton yield under rainfed condition besides holding higher moisture (Anon., 1986). Sowing as rainfed cotton, utilizing upplemental irrigation strategies or modified row configurations (e.g. skip rows) to enhance crop access to soil moisture, offer significant insurance against losses in both yield and quality in those regions and years where rainfall is highly variable (Bange, *et al.* 2005). Opening of furrow after every rows of cotton between 30 to 45 days after sowing and spreads of crop residue mulch were found to be promising at Maharastra (Gir, *et al.* 2008).

5. Weed management

Climate change especially due to elevated CO_2 affects C_3 and C_4 species differently and leads to different combination of crop-weed interaction. Weeds have a greater genetic diversity than crops. Consequently, if a resource (light, water, nutrients or carbon dioxide) changes within the environment, it is more likely that weeds will show greater growth and reproductive response. It can be argued that many weed species have the C_4 photosynthetic pathway and therefore will show a smaller response to atmospheric CO_2 relative to C_3 crops. However, this argument does not consider the range of available C_3 and C_4 weeds present in any agronomic environment. Today, for all weed/crop competition studies where the photosynthetic pathway is the same, weed growth is favoured as CO_2 is increased due to increased plasticity of weeds. There are some studies (Ziska and Teasdale, 2000; Ziska, *et al.* 2004) that demonstrates a decline in chemical efficacy with raising CO_2. Dilution effect of glyphosate for controlling Canada thistle under elevated CO_2 has been reported (Ziska, *et al.* 2004). Biological control of pests by natural or manipulated means is likely to be affected by increasing atmospheric CO_2 and climate change. Climate as well as CO_2 could alter the efficacy of weed bio-control agents by potentially altering the development, morphology and reproduction of the target pest. Increasing atmospheric concentration of CO_2 can alter the growth and physiology of weed plants. These changes could alter herbicide efficacy, crop-weed interaction and weed management (Joao Paul Rafarti, *et al.* (2019) and these author reported that the efficacy of grass killer Cyhalofop butyl was reduced to 50 per cent under elevated CO_2.

Increasing temperatures may mean an expression of weeds into higher latitudes or higher altitudes. With an increase of 3ºC, many weeds which are not problematic today may become aggressive as reported for itch grass (Patterson, 1995). Weeds being major pests competing for basic inputs with crop and being aggressive have developed all strategies to combat change in climate than crop (Devendra, *et al.* 2009). Witch weed, a root parasite of corn, is limited at this time to the coastal plain of North and South Carolina and with an increase of temperature of 3ºC, it is speculated that this parasite could become established in the Corn Belt with disastrous consequences. Clearly any direct or indirect impacts from a changing climate will have a significant effect on chemical management. Changes in temperature, wind speed, soil moisture and atmospheric humidity can influence the effectiveness of applications. For example, drought can result in thicker cuticle development or increased leaf pubescence, with subsequent reductions in herbicide entry into the leaf and hence suitable herbicide formulations are to be standardized to make the herbicide enter into the target sites.

The literatures on impact of climate change on weeds clearly suggest that many weeds which are less problematic or sleeper weeds may become aggressive and trouble some weeds due to climatic change and also the weeds may extend their spread to newer areas and hence it is necessary to generate information on these crucial aspects in order to equip our selves to meet any challenges in weed management due to climate change.

5.1 Exhausting weed seed bank by stale seed bed technique

Cotton is sensitive to weed competition due to its slow initial growth and wider spacing. In recent years, Bt cotton which is high yielding and responsive to higher levels of inputs like fertilizers, irrigation etc., is grown under intensive cropping system, all these factors promote luxurious growth of weeds which grow more quickly than cotton and compete strongly for soil moisture, nutrients, light and space. The competitiveness of weeds will still become worst due to climate change and technique like stale seed bed in which seedbed which has been prepared and given a false start some weeks before the seed is due to be sown, any weed seeds in the bed will be encouraged to grow so that they can be raked out and killed before the actual cotton crop is sown. This technique reduces the number of weeds which have to be controlled when the cotton seedlings start to grow in the field. Stale seedbeds are established several weeks or months before planting. Nalayini et al 2016 obtained the best weed control efficiency of 85.2% at 35-40 DAS with stale seed bed technique and application of pendimethalin and glyphosate @ 1.0 kg ai.ha to kill the germinated and germinating weeds so that weed pressure could be reduced during the actual cotton growing period (Table 4).

Table 4: Weed count, weed dry matter production and weed control efficiency of *Bt* cotton + coriander system

Weed control treatments	Weed count* 35- 40 DAS	Weed DMP g/m^2 35- 40 DAS	WCE (%)
SSBT glyphosate 1.0 kg – HW (35-40 DAS)	132.3 (11.50)	54.4	54.1
SSBT pendimethalin 1.5 kg- HW (35-40 DAS)	40.6 (6.37)	21.5	81.9
SSBT pendimethalin 1.0 kg + glyphosate 1.0 kg - HW(35-40 DAS)	30.9 (5.56)	17.6	85.2
Pre emergence weed control with pendimethalin 1.5 kg - HW(35-40 DAS)	53.59 (7.32)	30.5	74.4
SSBT and manual removal of weeds (thrice, week before sowing, 15-20 DAS and 35-40 DAS)	44.95 (6.71)	118.5	74.8
Un weeded control	555.25 (15.97)	4.67	-
CD (P=0.05)	0.56		

*Figures in parenthesis are square root transformed values for statistical analysis

5.2. Weed smothering by leguminous cover crops

Cover crops play an important role in smothering the weeds and covering the exposed land under vegetative cover until the main crop establishes so that the late emerging weeds could not compete with main crop. In addition to weed control through physical obstruction and/or biochemical suppression, cover crops provide numerous environmental benefits that can promote long term sustainability of farm lands. Leguminous covers such as hairy vetch (Vicia villosa) increase plant – accessible soil nitrogen leading to increase in growth and yield of cotton (Sainju, *et al.* 2005). Cover crops also improve soil composition, conserve soil carbon, nitrogen and moisture content and enhance microbial activity (Hoffman and Regnier, 2006). The combined effect of exhausting weed seed bank by SSBT technique and *in situ* leguminous cover crops for weed smothering in cotton is a novel approach in sustainable weed management of irrigated cotton (Nalayini, *et al.* 2017). Sun hemp as cover crop recorded the highest net benefit in cotton (Blaise, *et al.* 2020).

5.3. Herbigation

Herbigation, an application of herbicides through irrigation water can be efficiently done through drip irrigation. Cotton crop is sensitive to weed competition during initial growth stages due to its slow growth and wider spacing. The pre-emergence herbicides can manage weeds only up to 30 DAS (Nalayini and Kandasamy, 2001) and controlling the late emerging weeds is really a challenge in cotton production. Application of post emergence herbicides to supplement pre-emergence treatments may give the desired season long weed control in cotton (Dadari and Kuchinda, 2004). As we have a very few selective herbicides for post emergence application in cotton, farmers perform several hand weeding and inter cultivation operations to control weeds which adds to the cost of production. Providing timely weed control becomes difficult, in case of heavy rains, the soils become sticky and wet and traffic ability is poor while in the dry soil, the surface becomes hard making inter-row cultivation difficult and also, non-availability of human labourers for weeding makes timely weed control difficult, tedious and costly affair. Repeated use of same herbicide or herbicides of same chemical class may result in development of herbicide resistance in weeds. Vargas and Wright, (2005) suggested rotating herbicides with different modes of action to delay the development of resistance in weeds. In India, herbicides are generally sprayed using knapsac sprayers. Application of post emergence herbicides through conventional spraying is difficult near the cotton crop and hence weeds which emerge close to cotton crop escape and the post emergence residual herbicides could be efficiently applied through drip system (herbigation) as reported by Nalayini, *et al.* (2013).

6. Inclusion of legume with Cotton for sustainability

Sustainable use of soil is among the global challenges of the twenty – first century. High external input based cotton cropping system has depleted soil organic matter/carbon (SOM/SOC) stocks and fertility of soils and overuse of N fertilizers lead to more emission of nitrous oxide. However, Nitrogen fertilization is indispensable component of current production practices, hence high emission by using inorganic nitrogen fertilizer use be restricted (ICAC, 2009). The mitigation strategies should aim to reduce quantum of inorganic inputs used in cotton production system. The integrated nutrient management system (INMS), nevertheless, remains the maintenance and possible improvement of soil fertility for sustained crop productivity on long term-basis (Roy and Ange, 1991), increasing yield @ 22% (Govil and Kaore, 1997) and aimed also to reduce inorganic (N fertilizer) input utilization. Legumes rotation can fix atmospheric N to an extent of 135-488 kg $ha^{-1.}$ It is estimated that cotton following a non-legume rotation crop required an application of 179 kg N ha^{-1}, while following the grain- and GM-legume system it required only 90 and 52 kg N ha^{-1} respectively (Rochester, *et al.* 2001). It is evident that application of FYM, green gram mulching, Glyricidia green foliage lopping and sun hemp as GM recorded 15-32% increase in yield over control and there was considerable build up of soil available nutrients following these (Blaise, *et al.* 2004). It is usually recommended that slow-release nitrogenous fertilizers be applied to cotton at the pre-flowering stage and that nitrogen be made readily available at flowering when the demand is high (ICAC, 2009).

Legumes are soil-amendment crops with strong benefits on soil health and must be an integral component of the farming systems (Hauggaard-Nielsen, *et al.* 2007) and legumes must be included within cropping system for soil health management (Binder and Wiek, 2007). The important benefits of legumes include soil restoration, increase in the SOC stock, improvement in N pool by BNF, and positive effects on the yield of succeeding crop. Thus, there is an enormous need to focus on the benefits of legumes and their role in the soil sustainability (Dhakal, *et al.* 2016). Legumes can decrease the energy footprint of cropping systems by reducing the need for N application and restoring the health of agro ecosystem (Giller, *et al.* 2009; Verma, *et al.* 2015).

While, Haber-Bosch industrial process produces 100 Tg reactive N per annum globally, the atmospheric N-fixed by agriculturally important legumes is about 50–65 Tg annually, with 5–6 Tg fixed by other legumes in ecosystems (IAASTD 2008). Thus, there is tremendous scope of growing legumes in the cropping system to save energy intensive inorganic nitrogen besides improving soil health and microbial biodiversity. Indian soils are not only thirsty but hungry as 67% of our soil comes under low inorganic carbon status necessitating the urgent need to improve them through growing of legumes in the cropping system, a study was conducted at CICR to improve the soil organic carbon status and to reduce the dependence on inorganic N fertilizers by introducing perennial legumes as alley cropping in the cotton – maize irrigated ecosystem of Coimbatore. *Desmanthus virgatus* has been identified as the most suitable perennial legume to be grown as alley cropping with cotton. This legume crop is fast growing, hardy to withstand drought, no pest and disease problem and amenable for multiple pruning. Alley cropping of perennial legume, *Desmanthus virgatus* with cotton improved the soil organic carbon status from 0.57 to 0.69% in 32 months and added about 20 t of biomass into the soil which translated to 625 kgN/ha and resulted in 8 Q/ha of additional seed cotton yield (Annon, 2018-19).

7. Bio inoculants

Nitrogen and phosphorus are the two major plant nutrients required for higher productivity in many field crops grown in our country as majority of Indian soils are either low or medium in status for these two nutrients. Although nutrients are supplied as fertilizers to soil, they get either fixed or lost by various processes. However, micro-organisms play a major role in nutrient recycling in soil for supplementing crop requirements of nutrients either by dissolution from the soil reserve or by fixation from the atmosphere. Pink pigmented facultative methylotrophs (PPFM) influences seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins. The efficiency of conventional bio-inoculants like *Azospirillum* and phosphorus solubilizing bacterium (PSB) can be enhanced by co inoculating with PPFM (Nalayini *et al.* 2004). The gradual reduction in the use of pesticides and fertilizers and greater use of the biological and genetic potential of plant and microbial species may alleviate the high demand of agro-chemicals in sustaining high production in agriculture. In cotton, *Azotobacter*and *Azospirillum*were found useful in effecting N economy. Studies revealed that *Azotobacter* inoculation along with 40 kg N ha^{-1} was similar to application of 60 kg N ha^{-1} (a saving of 20 kg N ha^{-1}, Anon. 1985). Though the N fixing potential ranged from 40-60 kg N $ha^{-1}y^{-1}$, yet saving to an extent of 20-40 kg N ha^{-1} y^{-1} was observed under field condition. The Pink pigmented facultative methylotrophs (PPFM) is an useful bioinoculant for cotton for yield improvement, compatible with conventional bioinoculants like *Azospirillum* and PSb, proved for S oxidation under in vitro condition and has good scope to be explored for cotton based system to reduce the dependence on chemical fertilizers. (Nalayini, *et al.* 2014).

8. High Density planting system

Many cotton producing countries like Brazil, China, Australia, Spain Uzbekistan, Argentina and Greece tested, proved and adopted narrow row planting system of cotton as tool to achieve higher productivity (Kranthi, 2013). Increased plant density would be beneficial to enhance cotton yield in lower fertility field. High density planting system has been suggested as an alternative strategy instead of conventional one to increase yield. Prevailing manual picking cost constitute about 30-40 per cent of total cost of cultivation, which necessitates machine picking, thus ultimately warranting high density planting system with compact genotypes for its suitability. Packages for High Density Planting System including genotypes, spacing, nutrient requirement, mechanical sowing, land shaping, canopy management and weed control were developed.

At Hisar, compact culture, H1465 exhibited superior performance; However, in Sriganganagar, the highest seed cotton yield was recorded with RS2718. Amongst spacing tested, 67.5 X 20 cm and 67.5 X 15 cm produced high yield at Hisar and Sriganganagar, respectively. High density planting of 60 cm between rows and 10 cm between plants recorded significantly higher seed cotton yield in both locations of Rahuri and Surat. Nutrient response was significant at Surat but not at Rahuri.(AICRP on Cotton, 2017). High density planting system, in the Indian context, provided 25-30% higher yield over recommended spacing on shallow to medium deep soils under rainfed conditions (Venugopalan, *et al.* 2013). The HDPS trials conducted at Coimbatore in 2014-15 revealed that conventional planting registered mean seed cotton yield of 18.9 q/ha and mean net return of Rs 24567/ha (Table 5.). However planting under HDPS registered mean seed cotton yield of 30.7 q/ha and mean net return of Rs 50301/ha which were 62.4 and 104.8 per cent higher than conventional planting, respectively (TMC, 2015).

Under deficit irrigation, HDPS gave 9.1 to 17 percent enhanced seed cotton yield and 9.3 to16.8 % higher irrigation water use efficiency than low or medium plant density (Zhen and Dong, 2016) and higher yield of HDPS under deficit irrigation was due to higher biomass production and harvest index.

Table 5: Seed cotton yield (q/ha) and economic return (Rs/Ha) as influenced by HDPS cotton

Treatments	Seed cotton yield (q/ha)			Gross return(Rs/ha)			Net Return (Rs/ha)		
	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean
HDPS (60x10 cm)	32.4	29	30.7	111262	99891	105 576	53495	47108	50301
Conventional (75x45 cm)	20.2	17.6	18.9	69665	60848	65257	26695	22438	24567

9. Multitier Cropping

The risk and uncertainty imposed by climate change could be managed by intercropping and multi-tier cropping in cotton. Reilly, *et al.* (2001) and Butt, *et al.* (2006) found that modified crop mix, and land use are all potential adaptation to climate. Multi-tier vegetable intercropping, with short duration vegetables like coriander, radish, vegetable cowpea, cluster bean and beet root provide a suitable multi-tier combination along with cotton. Periodic and early harvest of intercrops resulted in less competition within the component of multi-tier crops leading to yield equal to sole cotton. Per hectare gross return of Rs. 1, 15,000, net return of Rs. 75,000, B: C ratio of 2.9, land equivalent ratio of 1.7, diversity index of 3.5 and per day return of Rs. 500 were realized with MULTI-TIER system involving radish, beet root, coriander with cotton under irrigated condition as reported by Sankaranarayanan, *et al.* (2007). Pulses like soybean, black gram, and green gram, were found promising in many situations for intercropping with cotton (Sankaranarayanan, 2010).

10. Conservation agriculture technique

Conservation agriculture practices can contribute to making agricultural systems more resilient to climate change. In many cases, conservation agriculture has been proven to reduce farming systems' greenhouse gas emissions and enhance their role as carbon sinks. In cotton, while using poly mulch, zero tilled rotation maize performed as good as the first crop of cotton (Nalayini, *et al.* 2009 with all the benefits of moisture conservation, weed control and reduced tillage with a huge saving of energy toward field preparation for rotation crops. Majority of crop residues are burnt in cotton based cropping system (*eg.* cotton-wheat in North Zone) resulting in emission of green house gases, in addition to loss of N, P, S and B. On the other hand, restoration of soil fertility is possible by incorporating the crop residues since the practice itself conserves/sequesters carbon as residue having wider C/N ratio possibly leaves more carbon in the soil. Study revealed that incorporation of cotton and wheat residues improved the productivity of these crops at Sirsa, Sriganganagar and Ludhiana in the North zone since improvements in the soil fertility might stabilize long-term yields. At Coimbatore , an integration of organics *viz.,* FYM @ 5t ha^{-1} (15 days before planting), cotton residues @ 2.5 t ha^{-1} (30 days before sowing) and sun hemp seeded @ 15 kg ha^{-1} simultaneously in inter-rows of cotton as GM and buried at 45 DAP produced significantly higher seed cotton yield and lower pest population over the recommended dose of fertilizers (Praharaj, *et al.* 2004).

Increasing temperature could accelerate decomposition of soil organic matter, releasing stored soil carbon into atmosphere (Knorr, *et al.* 2005; Fang, *et al.* 2005; Smith, *et al.* 2005). Low tillage practices are an example of win-win technology that reduces soil temperature in tropic, soil erosion and the use of fossils fuels and also act as mitigation strategy in cotton production system.

Adaptability of Cotton species to climate change

India is the unique country where all the four species of cotton are being commercially grown in some of the states. Studies on the effect of temper-ature in different genotypes (Reddy, *et al.* 1992) revealed that pima cotton *(Gossypium barbadense)* (Extra Long Staple) was found to be more sensitive to higher temperatures than the delta type of cotton plants *(G. hirsutum)*. High temperature sensitivity by producing no fruiting branches at 40/32°C, fewer branches at 35/27°C and more branches at 30/22°C, whereas the *G. hirsutum* plants produced the same number of fruiting branches in all these temperatures. Pima cotton exhibited greater damage to their reproductive structures at higher temperatures than the *hirsutum* cotton type. The study suggests that high temperature by climate change may affect the ELS cotton production.

Irregular and erratic distribution is expected by climate change; thus may result into wet spell and dry spell leads to water logging and drought. While considering the productivity of different species of cotton under rainfed condition, high rainfall year normally favors *hirsutum* over *arboreum / herbaceum* cotton and the reverse is true for a low or scanty rainfall year. For maximum utilization of rainfall under both high and low rainfall situation by inter cropping of *arboreum*, *hirsutum and herbaceum is* one of the options. Species intercropping studies at Kovilpatti (TN) revealed that planting of *G. arboreum* (25%) + *G. herbaceum* (25%) + *G. hirsutum* (50%) found to record higher yield and stability among the different proportions and sole cropping both under the high and low rainfall situations. Krishnasamy*et al.* (1995) reported that *arboreum* genotypes registered higher seed cotton yield (430 kg ha^{-1}) with low and erratic rainfall (268 mm) in comparison to hirsutum cotton (262 kg ha^{-1}) at Kovilpatti (TN). Bhatade, *et al.* (2008) found that Gossypium *arboreumviz.,* J. Tapti and NA-398 were found to be the best performers compared to *hirsutum* hybrids/varieties (NHH-44 /NH-545). Monoculture of *G. arboreum* cotton, out-yielded monoculture of *G. hirsutum* cotton in low rainfall years (nine) out of eleven years except in years of heavy rainfall at Nagpur (Venugopalan and Pundarikakshudu, 2009).

High temperature may result into high evapotranspiration, less soil moisture and salinity in semi arid condition. Gopalakrishnan (2010) reported that *G. herbaceum* cotton is tolerant to salinity, drought and well adapted to marginal soils which are as bad as desert sands. Large scale evaluation of ninety two cotton genotype comprising *G. hirsutum* as well as *G.herbaceum*lines was carried out at Raichur, Karnataka by providing protective irrigation. As a group, *G.herbaceum* genotypes performed better than *G.hirsutum* genotypes and clear indication was evident about suitability of *G.herbaceum* genotypes in situations wherein only limited irrigation was possible. *G.herbaceum (*RAHS-14) exhibited higher level of tolerance to salinity stress and recorded highest seed cotton yield .The review summarized that *barbadence* may be sensitive then *hirsutum* and diploid cotton perform better under climate aberration posed by climate change.

Conclusion

Various technological options discussed and reviewed in this chapter are the climate smart technologies for water saving, energy saving and better carbon sequestration, having potential to enhance the seed cotton yield with sustainability and for climate resilient cotton farming and need of the day to reduce GHG into atmosphere and save the planet from further degradation.

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