Gossypol: Toxicity, Importance and Availability

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***Abstract*— Gossypol is a polyphenolic highly toxic compound found mainly in cotton plant. Cotton seed is a rich source of oil and of good quality protein. Gossypol's presence in cotton seed meal makes the meal unfit for consumption both for human being and animal feed due to its toxicity. In order to supplement protein content of the feed, defatted cotton seed meal is used in regulated quantities only. The consumption of cottonseed in excess quantities may lead to severe health problems and even lead to the death of the animal. Similarily, the raw cottonseed oil is also unsuitable in food application. The oil is usable after thorough refining only so that the gossypol is removed. On the other hand, gossypol when present in the cotton plant is reported to work as a natural defense system to protect the plant from insects, pests and diseases. Additionally, gossypol has been found to be useful as a male contraceptive in China. It also possess antiseptic, antiviral, antimalerial, antiatoptotic and antipsoritic activity. Recent interest is shown by scientists in gossypol as an anti-cancer drug. Gossypol is reported to be a precious compound due to its diversified uses in medicine, pharmaceutical, plastic, pesticide and various other applications. The present paper enumerates availability, chemical properties, toxicity and uses associated with gossypol.**

***Index Terms*— Gossypol, Cottonseed, Extraction, Drug.**

I. INTRODUCTION

Cotton cultivation is carried out to meet the basic requirement of fabrics for the masses. The plant was discovered some 5000 years ago in Mexico region. Four species of cotton namely G hirsutum, G barbadense, G. arborium and G. herbaceum are cultivated globally and a number of other specie grow wildly in the nature. The indigenous species, Gossypium hirsutum is the most planted species of cotton covering approximately 90% coton production worldwide. More than 20 million farmers grow cotton globally for fulfilling the need of cotton fabrics and cottonseed is left behind after ginning the cotton balls. It had very little commercial value for many centuries as it was used mainly as the fertilizer. but today it has gained importance due to its nutritive content. The other parts of the cotton plant like cotton root bark, leaves and stem also contain gossypol. Gossypolprotects the plant from insects and pests naturally. In addition to the cotton plant, gossypol has been reported [1] to be present in some other species of Malvaceas as shown in Table-1

Table-1 Gossypol content mg/100g dried sample on ten species of Malvaceas[1]

Species	**Gossypol in Seeds mg/100g**	**Gossypol in Leaves mg/100g**
Anoda cristata L. Schltdl	27.54	3.52
Hampea integerrina Schltdl	1180.00	0.00
Hibicus clypeatus L Shesh Tendal	4.37	0.00
Hibicus rosa-sinensis L.Shesh Tendal(Gurhal,China Shoe Flower)	**2.05**	1.87
Hibicus sabdariffa L.Shesh Tendal (Patva)	**0.00**	0.00
Malvaiicus aboreus ICav Schltdl	42.69	0.00
Malvacicus arborues II Cav Schltdl	0.00	0.00
Malvavicus arboreus Cav Schltdl	4.47	0.75
Pavonia Schideana Stend.J.	3.33	0.00
Gossypium Hirsitium L.(American Cotton)	847.00	297.00

Gossypol is the predominant yellow pigment and is found to be the major toxic ingredient present in cotton plant (Gossypium spp.) along with some other polyphenolic pigments. The name gossypol is derived from the plant's scientific name (*Gossypium*) combined with active phenol group ending with "ol" [1]. These pigments are contained within small discrete structures called pigment glands of size 50-400 microns, (Figure-1)which are distributed in all other parts of the plant like seed, root bark and leaves in different concentrations & work as a natural defense system for the cotton plant. Gossypol is present in free form while being in the glands but as soon as these glands are ruptured, the gossypol comes in contact with the proteinous part of the seed and gets complexed with the protein to form bound gossypol. The complexing increases with the rise in temperature. Both these forms (free and bound) put together is called total gossypol. Free gossypol has been found to be highly toxic where as the gossypol bound with proteinous substances is not toxic as it is not available freely in the stomach. Gossypol content of cottonseeds varies from traces to 6% depending upon the plant, species, variety and environmental factors like climate, soil type and fertilizer [2].

Manuscript received Sep 17, 2015
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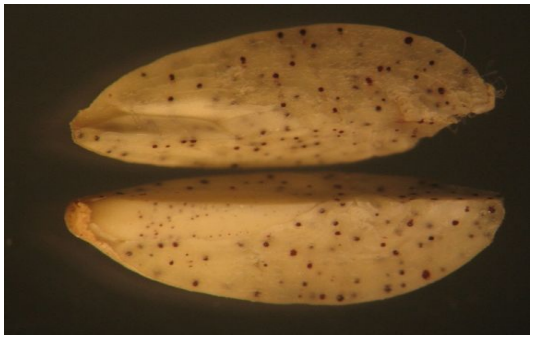

Figure-1 Gossypol glands in cross-section of cottonseed meal

Global production of cotton is approx. 120 Million Bales (1 Bale=170 Kg), China is the largest producer of cotton whereas India continues to be second in cotton production from 2006-07 after surpassing the cotton production of United State. Production of top 5 cotton production countries along with total world production of 5 years is presented in table 2.

II. AVAILABILITY OF GOSSYPOL FROM COTTON PLANT

It has been reported that for every Kg of cotton, the plant produces approx. 1.65 Kg. of the seed. With a global production of approx. 119 million bales or 20.23 M.M.T. (Million Metric Tons) of cotton and (20.23X1.65) =33.38 MMT of cottonseed. Considering an average of 1.8% gossypol in cottonseed with an experimental recovery of 92.4% [3] we may get approx 0.55 Million Tons of gossypol can be obtained from the seeds. The other major source of gossypol in cotton plant is cotton root bark which is reported to contain up to 1.8% gossypol. Experimental recovery of pure gossypol from the

Table:2	**Cotton Production of top 5 countries and world in Million Bales**				
Country	**2011/12**	**2012/13**	**2013/14**	**2014/15**	**2015/16 July**
China	34	35	32.8	30	27
India	29	28.5	31	29.5	29.5
U.S.A.	15.6	17.3	12.9	16.3	14.5
Pakistan	10.6	9.3	9.5	10.6	10
Brazil	8.7	6	8	7	6.8
World (Total)	127.4	123.8	120.5	119	111.5
Source: Monthly Economic Letter | August 2015. Cotton Incorporated					

sample of root bark was reported to be 0.88%[4][49]. Roycee et. al.[5] reported that one acre of cotton area yields about 150 Kg of dry root from which about 1.3 Kg of pure gossypol can be recovered. Global area under cotton farming is around 35 Million Hectare (1Hectare=2.47Acre) from which we can have around 0.114 MMT (1.32X2.47X35=114Kg) gossypol per annum from cotton root bark. The cotton leaves are reported to contain up to 0.3% gossypol but no data is available on its extraction rate and quantity. In stem negligible amount of gossypol is reported thus, putting together the seeds and root bark we can have an appreciable amount of 0.66 MMT gossypol from cotton plant alone annually.

III. PROPERTIES OF GOSSYPOL

Gossypol is yellow colored powder with molecular formula $C_{30}H_{30}O_8$ having Molecular wt. 518.56. It is soluble in many organic solvents like acetone, ether, hexane, ethanol, isopropanol etc. but is insoluble in water.

The chemical structure of gossypol was first derived in 1938 by Adams after extensive studies on its properties and reaction. Twenty years later Edwards confirmed Adams' structural formula and he was first to synthesize gossypol[6]. Gossypol is a symmetrical molecule which has restricted rotation around the bi-napthyl bond and is reported to exhibit atropisomerism [7]. Chemical structure of gossypol is shown below figure 2

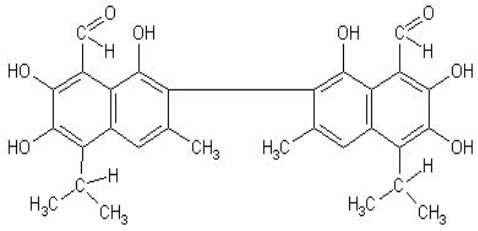

Figure 2 Chemical structure of gossypol

Gossypol exists in the form of two atropisomers {(+) and (-) isomer}. In commercial cotton variety (G. Hirsutum) the ratio of (+) to (-) gossypol is approximately 3:2. The (-) form of gossypol is reported to be more active and useful form. Out of all the cotton species, only Gossypium Barbadense has been reported to produces an excess of (-) gossypol with the ratio of (+) to (-) as 2:3. [8][23] Figure 2.1

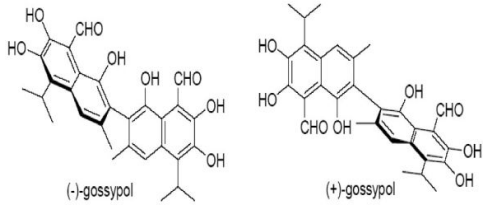

Figure : 2.1 Atrop isomers (+) and (-) Gossypol

Tautomerism in gossypol

Pure crystallized gossypol has been found to have distinct melting point depending upon the solvent used for its extraction. Adams (1938) found gossypol to exist in three tautomeric forms as shown below.

a) Gossypol crystallized from ether melts at 184^0

b) Gossypol crystallized from chloroform melts at 199^0C

c) Gossypol crystallized from Skelly-B (a high boiling petroleum ether) melts at 214^0C. Figure 2.5.

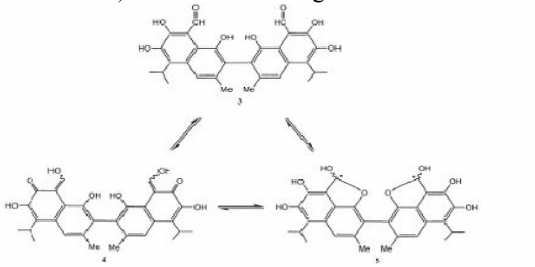

Figure 2.5: Symmetrical tautometric form of gossypol : dialdehyde 3, diketone 4, and dilactol 5

These tautomer have been found to have reversible transformation which can be seen by taking crystals formed from one of these solvents and re-dissolving and re-crystallizing them from a different solvent. The resulting crystal melts at the temperature corresponding to the new solvent. Although having different melting points all these crystals give the same chemical reactions [8].

IV. GOSSYPOL TOXICITY

Cottonseed meal is a rich source of high quality protein but is not safe for consumption as a human food or animal feed due to the presence of toxic "Gossypol" which is a polyphenolic aldehyde that permeates cells and acts as an inhibitor for several dehydrogenase enzymes including protein kinase C [9]. It has also been said to cause low potassium levels and thus cause temporary paralysis. The studies also discovered an abnormally high rate of hypokalemia- a low blood potassium level, resulting into kidney malfunction. It also develop symptoms of fatigue, muscle weakness, leading to paralysis among patients. In addition, about 7% of patients reported effects on their digestive systems and about 12% increased fatigue. It was observed that after stopping the use of gossypol and taking potassium supplements most patients recovered from the illness. As regards animals, gossypol primarily attacks the heart and liver [10], the reproductive tract, abomasums and kidney of the simple stomached (monogastric) animals such as pigs are also affected .due to gossypol toxicity. In early 1900's ruminants were also considered subject to gossypol toxicity in the form of "Cottonseed meal ((CSM)) injury" however in 1930's it had been demonstrated that the CSM injury was attributable to Vitamin-A deficiency. Gossypol toxicity has not been reported in mature ruminants consuming cottonseed meal or cottonseed. Ruminants such as cattle and sheep can tolerate higher levels of free gossypol because gossypol binds to protein in the rumen whereas young calves and lambs are quite susceptible to gossypol toxicity as their rumen is not fully functional and is unable to bind as much free gossypol as an adult. However, increased feed intake by high producing dairy cows consuming rations containing CSM could adversely affect the protective function of rumen in preventing gossypol toxicity.[10] Swine, guinea pigs and rabbits are reported to be most sensitive towards gossypol, cats and dogs have intermediate sensitivity and poultry, mice and rats have low sensitivity. Table-3 indicates the level of gossypol recommended in some of the animals and human food [11]

Table-3

Animals	Maximum gossypol allowed in ration
Swine,	100 ppm
Calves and Lambs less than 4 months old	100ppm
Adult Cattle	800ppm
FDA norms for Human Food	450 ppm

V. SYMPTOMS IN ANIMALS DUE TO GOSSYPOL TOXICITY

Gossypol toxicity has been indicated in two types of clinical syndromes particularly in young animals due to its affect on the heart [11]. One syndrome of sudden death (resembling a heart attack) has frequently been reported in calves and lambs. Generally healthy looking animals with good appetite and apparently one of the best in group, are suddenly found dead. Calves on cottonseed diet may die suddenly during or right after transport because of high stress. The other syndrome is one of chronic labored breathing which resembles pneumonia. Due to effect on heart, the lungs will fill up with fluid and breathing becomes very difficult. Since it is not an infection, these animals do not respond to antibiotics. Animals may be depressed, go off the feed and may die gradually. However, this is in contrast to the animals appearing looking healthy but die suddenly during or after transportation. Adult dairy cattle have exhibited symptoms of weakness, depression loss of appetite difficult breathing, blood in urine, inflammation of the intestines and reproductive problem. The toxic effects of gossypol on human being have largely been assumed on the basis of its effects on animals.[12][13][14]

VI. IMPORTANCE & USES OF GOSSYPOL

In spite of its toxicity on one side, gossypol on the other side is valuable and is gaining importance due to its diversified uses. Starting from 1861 all the early researchers thought an excellent use of gossypol and its brightly colored derivatives as fabric dyes [2]. Subsequently, gossypol was reported to be one of the strongest antioxidants found in nature. (But it was never used in food products because of a belief in its toxicity). Although, Hove and Hove reported that in non harmful concentration, it is still a very effective antioxidant. Royce 1933[5] reported on the possible use of gossypol as an antioxidant in the petroleum and rubber industry. The Japanese in 1954 reported its use as a stabilizer for vitamin A in the products. Researchers in USSR have also done extensive research on all aspects of gossypol and have found gossypol to be an effective antioxidant-stabilizer for PVC and Polypropylene. They have also reported its use as a stabilizer in cement mixtures for road foundations and in spackling, where it increases the life of road and cold resistance and decreases water absorption. There are numerous uses of gossypol in drilling muds used in petroleum industry, where it is reported to increase heat stability and suppress viscosity and water loss. In 1970's the use of gossypol was patented (Naval Weapons Center in China Lake, California) to extend batch mixing time and act as an antioxidant in rocket propellant formulations. The early investigators on gossypol had rightly suggested its possible use in pharmaceuticals. And now it is the area where it has gained utmost attention.[2][8]

6.1 As a Male Contraceptive: An accidental discovery of gossypol as a male contraceptive was reported in China by Liu who reported that "Wang village in Jiangsu, China did not have a single child birth for nearly 10 years between 1930's and 40's but before and after this period there seemed to be no birthing problem". He found that the villagers had shifted to crude cottonseed oil in their cooking due to poor economic conditions. Liu suggested that the gossypol content in oil might be the cause for infertility. In 1967 medical workers reported observance of the disease called "Burning Fever"

caused by the ingestion of crude cottonseed oil and one of the symptoms of this fever was infertility. In 1970's the Chinese govt. began researches on gossypol as a contraceptive to conclude that gossypol provided reliable contraception and could be taken orally as a tablet and did not change men's balance of hormones. In mid-1990s the Brazilian pharmaceutical company Hebron announced plans to market a low dose gossypol pill called "*NOFERTIL*" but the pill could not be introduced into the market due to unacceptably high rates of permanent infertility. But the research still continues for using it as an alternative to vasectomy in Australia, Brazil, Chile, China, Nigeria etc.[15]

6.2 As a Herbal Medicine: One of the earliest reported medical use of gossypol occurred in 1872 when Wayne described the use of cotton root bark extract (rich in gossypol) to promote or assist menstrual flow and to prevent hemorrhaging. While it was used to stimulate menstrual flow and make child birth easier, cotton root bark was also taken as an abortifacient to induce miscarriage. Chewing of root was said to stimulate the sexual organs (called as an aphrodisiac). Contemporary uses of cotton root bark cover nearly every aspect of female reproductive system. Numerous other uses are listed in such PDR (Physician's Desk Reference) for Herbal Medicines, the 1998 book based on the findings of Germany's Commission E. The European group's findings about herbal remedies were published in a 1997 monograph. Murthy [2] discovered that gossypol effectively inhibits egg implantation. Researchers in 1984-85 found that gossypol and its derivatives have been shown to have significant antimicrobial activity as well as wound healing effects and may find applications in ointments. It also exhibits anti malarial activity and is reported to kill viruses such as herps. Gossypol was reported to be a very powerful inhibitor of "CHAGAS" disease (a deadly Central and South American parasitic disease). Its use as an appetite suppressant has also been reported [2]

6.3 As a Traditional Medicine: In traditional medicine, gossypol was reported to be used in Mexico for scalp infection, dysentery, gonorrhea and as an antiseptic. In addition, the gossypol extracted from cottonseed is long known to possess antiviral, antipsoriasis, anti keratititus and neoplastic activity, due to which it finds its use in the field of medicine. The antimicrobial and pharmacological activities of gossypol is of increasing emphasis in recent years The investigations are being carried out on crude gossypol and some of its derivatives for their bioactive potentials against Gram +ve (Staphylococcus aureus) and Gram –ve (Escherichia Coli) pathogenic bacteria.[1]

6.4 As an Anti Cancer Drug: Recently, new interest has developed in gossypol as an anticancer drug. A study in Ohio in 1996 reported that gossypol is a potent inhibitor of prostate cancer cell growth. Tests on breast cancer cells have also shown that (-) gossypol is more potent inhibitor of cancerous breast cell growth. Recently gossypol has been shown to possess anticancer properties both in vitro and in vivo. The natural form of gossypol has been extensively tested in humans and is well tolerated for long term use. It is currently believed that gossypol in itself does not kill cancerous cells but it changes the chemistry within the cell and makes it more susceptible to traditional chemotherapy drug. If we can use the more active form (-) gossypol correctly and in combination with radiation or chemotherapy – gossypol may soon find its new role in the fight against cancer [16]. It's proapoptotic properties reported earlier are probably due to the regulation of Bax and Bc12. It also reversibly inhibits calcineurin and binds to calmodulin. It is also reported to inhibit replication of the HIV-1 virus [9] [48].

6.5 As an Insecticide: Low concentrations of gossypol and other cotton phyllophase toxins from cotton seed have been reported to improve the effectiveness of insecticidal agents against social insects including cockroaches, and particularly termites and ants. Furthermore, the insecticidal agents who are ineffective in control of social insects alone may be effective when used in conjunction with the cotton toxins [2]. These diversified uses of gossypol have made it a very precious chemical. A look of recent catalogues of major chemical supplier reveals that gossypol in purest form is many costlier as compared to the current price of gold [18].

VII. SEPARATION/ INACTIVATION OF GOSSYPOL

Different methods have been tried researchers to separate gossypol from the cottonseed or detoxify the seed meal by different methods. Efforts have also been made to develop glandless variety of coton plant which is free from gossypol. Some common approaches are enumerated below.

7.1 By Solvent Extraction Process: The solvent extraction process is reported to be the most efficient technique for removal of gossypol from cottonseed [4]. The efficiency of gossypol extraction depends upon the preparation of the seed prior to extraction, which includes the particle size, temperature, mode of operation (batch or continuous and co-current or counter-current operations) etc. The choice of solvent is based on solubility of gossypol in the selected solvent, cost and safety. In case of gossypol extraction from cottonseed the selection of a solvent is also done on the basis of its working efficiency at low temperature (preferably below 50°C) in order to prevent its deactivation by complexing with lysine present in the seed. Considerable work has been carried out using both polar and non-polar solvents for separation of gossypol from the cotton seed. Some of them are given below.

Smith [18] reported that cottonseed kernels containing 0.7 to 1.0% gossypol yields 0.4-0.6% crude gossypol when extracted with ethyl ether (i.e.60-65% yield) without previously extracting the oil with petroleum ether. In purifying the crude gossypol 80-85% yield was obtained as acetate for each crystallization.

Dachery [19] reported that since gossypol is more soluble in polar rather than non-polar solvents, a polar solvent-water pair should prove more effective. Various solvents tried by him were methanol, acetone, ethanol, isopropanol, butanone trichloro-ethylene, perchloro ethylene along with 10 different solvent water pairs. Out of these, butanone - water pair was reported to be most effective extractant.

Chemenko et. al. [20] reported that when the flakes are first steeped in concentrated miscilla and are then treated with pure hexane, it is possible to extract 60-65% of gossypol. It is also shown that the bulk of gossypol is extracted in the first half hour.

Rama [21] extracted cottonseed flakes with one of the following solvents (A) hexane, (B) 1:1 mixture of 85% isopropyl alcohol and hexane and (C) acetone, followed by 1:1 mixture of isopropanol and hexane. Out of these,

cottonseed meal with solvent (B) had been reported to contain the lowest free gossypol 0.069%

Yuan et. al. [22] discussed large scale extraction of gossypol from cottonseed through the use of 75-78% liquid acetone solution as well as some improvements on the methods used for purification of gossypol.

Hron et. al.[23] extracted gossypol from cottonseed meal using acetone as solvent. Acetone is an excellent solvent for vegetable oil. It has been suggested for use in combination with hexane and water to extract gossypol from cottonseed. A major reason reported for non acceptance of acetone as a solvent is that it usually impart a very objectionable "catty" odor.

US Patents [24] indicates recovery of gossypol from cottonseed gums by refluxing with methyl ethyl ketone having phosphoric acid.

US patent [25] states the extraction of gossypol from cottonseed by using a solvent solution which includes (a) a water miscible organic solvent (b) water (c) a suitable acid of medium strength to produce cottonseed meal with reduced gossypol.

Hron et. al. [26] tried Ethanol (EtOH) due to GRAS (Generally Recognized As Safe) rating as solvent and indicated that atleast 50% of total gossypol, 90% of aflatoxin and oil can be extracted in a two stage extraction process.firstly at 22-25^{o}C to remove gossypol and in second stage the extraction was done at 78^{o}C to remove oil, aflatoxin and some remaining gossypol.

Bhowmick [27] showed improved utilization of cottonseed meal by isopropanol extraction. The main advantage of IPA was reported to be its greater solvency and ability to detoxify the oil bearing material. Moreover, its other advantage is its greatly reduced fire hazards.

Kuk et. al. [28] extracted cottonseed with mixtures of acetone and hexane with concentration of acetone varying between 10-75%. Adding acetone <25% increased the extraction of free and total gossypol from cottonseed flakes. More than 80% of free gossypol was reported to be removed by 10/90 acetone/hexane mixture without catty odor in the cottonseed meal.

Saxena et. al [29] reported that up to 61% gossypol could by extracted from cottonseed meal using green solvent, pure ethanol Saxena et. al. [3] reported that up to 92.4% gossypol could be extracted by acidifying ethanol by oxalic acid.

7.2 By Gland Separation Process: The other process of gossypol separation was by development of process that remove intact pigment glands without adversely affecting the protein fraction namely air classification [30]. Boatner [6] way back in (1946) tried gland floatation process where the separation was done by density difference between glands and other material. The gland being lighter, floats on the top and can be skimmed off. Cherry [31] tried liquid cyclone process using Methylene chloride, In all these methods the separation of gossypol was not found to be complete as it still contained some other components along with gossypol.

7.3 By Pretreatment to Reduce Gossypol: It has been reported that processing by heat treatment [32] and extrusion process [33] can reduce free gossypol concentrations in the cottonseed meal. Another method of supplementation with ferric sulfate reduces free gossypol concentrations in food due to ferric sulfate binding with reactive groups from gossypol, which forms a conjugate. The recommendation for supplementation is 1 mol of gossypol for each mol of iron, which could increase the maximum concentration of gossypol from 50 to 150 ppm for laying birds and from 100 ppm to 400 ppm for pigs and poultry [34]. Additional nutrients may be used for dietary supplementation to reduce gossypol availability. Supplementing the diet with 1 mg of sodium selenite per day in adult sheep reduced the gossypol toxicity affecting semen quality [35]. Dietary vitamin E supplementation at 4000 IU/bull/day also reversed the negative [14]

7.4 By Irradiation: Radiation treatment using gamma [36, 37] or electron beam irradiation may reduce free gossypol concentrations. In fact, gossypol irradiation reduced *in vitro* pro oxidative activity and embryo toxicity in mice [38]. The mechanism for gossypol destruction through radiation is unknown, but it has been speculated that gossypol molecule aggregation, gossypol cross-linking with other molecules, and gossypol molecule fragmentation or breakdown may produce such destruction [39]

7.5 By Fermentation Process: Some fung us may reduce free gossypol concentrations in cottonseed meal by fermentation, including *Aspergillus niger* [41], *Aspergillus oryzae* [42], *Candida tropicalis* , *Saccharomyces cerevisiae* and *Geotrichum candidum* [43]. The use of fermented cottonseed meal to feed animals seems to be safe, solid substrate fermentation [44][45]. However, while these microorganisms could be used to reduce free gossypol concentration in cottonseed meal, they are not currently commercially used.

7.6 By Developing Gland Less Cotton Plant: Biotechnologists at Texas University have tried to develop glandless cotton plant i.e. the plant without gossypol in it by genetic transform. Although the plant was developed in the lab but it failed bitterly on the field trials as the crop could not survive in the absence of gossypol, a natural insecticide[46][47]. Now, further trials are continued using RNAi technology so that cottonseeds may be glandless whereas the other parts of the plant like root stem and leaves will have gossypol, to protect the plant from pest and insects. Toxicity from Cottonseed Products [49]

CONCLUSION

Gossypol is a highly toxic compound when present in cottonseed and makes it unfit for consumption, both as food and feed. After separation of gossypol from cottonseed in purest form, it becomes highly precious compound due to its diversified uses in medicine, insecticide, pharmaceutical and plastic industry. The gossypol can be made available from the cottonseed, root and leaves of cotton plant up to 0.664 MMT annually. Presently, high purity gossypol is costlier than the price of gold in the international market [50]. At the same time after extraction of gossypol from the seed, the seed meal becomes a rich source of good quality protein which can find application in energy food for protein enrichment. Although a lot of work has been carried out for extraction and purification of gossypol but the need for developing a commercially viable method to extract gossypol still exists to make this precious commodity available to masses at affordable prices.

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