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GENETICALLY MODIFIED CROPS

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Genetic Modification and Genetically Modified Organisms (GMOs)

GMOs are organisms which carry laboratory-made semisynthetic gene constructs, artificially introduced into the genome (the repository of specific hereditary materials of an organism), following the techniques of genetic engineering (GE). GE enables transfer of a gene or genes across all biological barriers, species, genus, order, phyla, etc. without the necessity of any sexual compatibility. Technically, all GMOs are artificially produced mutants with alien genes and are therefore different from naturally produced pure lines or hybrids which are usually obtained from intraspecific mating of compatible partners. The transgene may be accompanied by marker genes and will have their own alien viral promoter / regulator genes.

The transgene insertion in the from of a semisynthetic gene construct along with promoter / regulator and marker genes into the genome is as yet random and imprecise in terms of location, regulation and stability. All GMOs are large mutations with manifold consequences which would always run the risk of elimination from the genome. Further, the classical concept of gene expression on which genetic control of manifold characters depend, has now given way to the concept of fluid genome with intergene communications along with strong epigenetic effects in particular. These observations imply that current genetic manipulations for the creation of GMOs are imprecise, unstable and risky from health, ecological and environmental viewpoints.

Genetically modified crops in commercial cultivation :

When the genetic modification is carried out with crop plants the resultant plants are termed genetically modified crops (GMCs). All GMCs now under commercial cultivation are patented products (protected under intellectual property rights), developed and owned by multinational corporations, with the US-based Monsanto Company as the undisputed leader (also famous at home and abroad for its aggressive marketing strategies).

Three important commercial crops, cotton, soybean and maize, the latter two with principal uses as livestock feed and a relatively minor one, canola (oilseed rape), mainly confined to a few countries like Canada), have large acreages covering 134 million hectares of global crops. In the USA alone – the heartland of GM crops, the three major GM crops between them cover 85-91 per cent of the total area planted with these crops (171 million acres, 68.4 million ha) with herbicide tolerant (HT) crops covering 91% of soybean, 68% of corn and 71% of cotton acreages; insect tolerant Bt crops cover 65% of cotton and 63% of corn areas (no Bt in soybean); stacked (HT+Bt) GM crops cover 48% of cotton and 46% of corn areas. Elsewhere, the main emphasis is on HT soybean grown quite extensively in Argentina, Paraguay and Brazil; India and China have converted large areas under cotton to Bt cotton.

Introduction of alien genes from two soil bacteria, *Agrobacterium tumefaciens* and *Bacillus thuringiensis* would impart tolerance to herbicide application and resistance to a group of lepidopteran insects, respectively. While herbicide tolerance (HT) is attributed to a gene derived from the soil bacteria, *Agrobacterum tumefaciens* that would code for the enzyme EPSPS (5-enolpyruvylshikmate-phosphate synthetase), which is responsible for metabolizing and breaking down the herbicide glyphosate (gene amplification), the insect resistance (resistance to some lepidopteran insects) is attributed to one or more toxin-producing genes derived from the soil bacterium, *Bacillus thuringiensis* (abbreviated as Bt, hence such GM crops are known as Bt crops).

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Although the present commercial GM crops are either toxin-promoting (such as the HTs that promote use of toxic herbicide) or toxin-producing (such as Bt crops that produce cry toxins which are insecticidal biopesticides), the first commercial GM crop, a delayed ripening tomato, Flavr Savr (produced in1994), was withdrawn from the market by Monsanto within a short period because of health risks (publicly stated as commercially nonviable). Subsequently, none of the so-called sensational ones including the much touted Golden Rice of Syngenta, or experimentally successful drought and salt resistant crops, are yet to see the light of the day in terms of risk-free acceptability and commercial viability.

Problems with Large Scale Cultivation of GMCs

The large scale shift to GM crops has now brought with it still larger problems of continued rise in the population of insects pests that are resistant to Bt. The tarnished plant bug (TPB) has already devastated vast areas of Bt cotton in the USA. A similar situation is noted in India, where pink boll worm has become resistant to Bt, and mealy bug and other secondary insects have taken over. In China, the mirid bug has emerged as a serious secondary pest with outbreaks in multiple crops correlated with widespread adoption of Bt cotton.

A wide range of weeds that are tolerant to very large applications of herbicides so much so that very recently world's leading herbicide glyphosate (Monsanto's Roundup along with Roundup Ready soybean) has been almost fully rounded up and engulfed by nearly a dozen weeds some of which would eat up a couple of hundred times the recommended dose of the superhyped herbicide "as safe as table salt" (very correct as far as the resistant weeds are concerned but very toxic to soil biota and humans); the developer would now pay farmers in the USA 6 dollars per acre for herbicides of rival companies to combat the resistant weeds.

Monsanto pays farmers to spray crops with rival herbicides : According to a report on October 10, 2010 in the Des Moines Register, US farmers with large planting of Monsanto's Roundup Ready soybean or cotton, genetically engineered to withstand application of the company's Roundup herbicide (glyphosate) will get 6 dollars for each acre if at least two other herbicides (other than Roundup) are applied. That literally means that millions of acres across South and Mid West USA would get doused with Monsanto-subsidized poison (of utter humiliation!) of having to pay farmers to spray the herbicides of rival companies because of an abject failure of their much hyped package (RR crop + Roundup herbicide) and possibly a much bigger compensation burden (at least in the USA, where farmers will not leave it as such).

Roundup itself has long been facing challenges. Very importantly, a study by France's University of Caen in 2009 revealed that the herbicide's allegedly inert ingredients magnify glyphosate's toxic effects. According to the study, "the proprietary mixtures available on the market could cause cell damage and even death at levels commonly used on farm fields".

GM Soy: Neither safe nor sustainable : A group of international scientists released a report in September 2010 dealing with health and environmental hazards from the cultivation of genetically modified (GM) Roundup Ready soybean and the use of Roundup herbicide (glyphosate). The report, "GM Soy: Sustainable ? Responsible ?" highlights new research by Argentine government scientist, Professor Andres Carrasco (2010) which found that glyphosate causes malformations in frogs and chicken embryos at doses far lower than those used in agricultural spraying. The findings in the lab are compatible with the malformations observed in humans exposed to glyphosate during pregnancy. The report is released with testimonies of Argentine villagers whose lives have been radically disrupted by the cultivation of GM soy. In Argentina and Paraguay, doctors and residents have reported serious health effect from glyphosate spraying including high rates of birth defects as well as infertility, still births, miscarriages and cancer. Scientific studies have been collected in the new report that confirm links between exposure to glyphosate and premature births, miscarriages, cancer and damage to DNA and reproductive organ cells.

Global Promotion of GMOs as a Major Issue of the US Foreign Policy

The US Global Food Security Bill of 2009 sponsored by Richard Lugar, Robert Casey and 7 other US Senators in February 2009 is, "A bill to authorize appropriations for fiscal years 2010 through 2014 to provide assistance to foreign countries to promote food security, to stimulate rural economics and to improve emergency response to foods crisis, to amend the Foreign Assistance Act of 1961 and for other purposes".

However, the proposed amendment to the Foreign Assistance Act (FAA) of 1961 has proven controversial. It would include research on biotechnological advances appropriate to local ecological conditions, including genetically modified technology.

Widespread opposition to GM mandate in the US bill : In April 2010, 140 civil society groups, and scientists signed an open letter to US Senators urging them to "strip the GM mandate" from the Global Security

Act - the object to the clause effectively ear marking one agricultural technology, genetic modification, for billions of dollars in federal funding. US $ 7.7 billion goes with the bill, and no other farming methods are mentioned.

Not surprisingly, Monsanto has lobbied the hardest to support the bill. The US Company is the world's leader in the increasingly concentrated agricultural biotech industry, which is already subject to an anti-trust inquiry. The company is likely to benefit most with huge profit for its patented products (both GM seeds and associated pesticides).

Pressure to dump GM crops to unwilling countries in Africa: Mariam Mayet of the African Centre for Biosafety based in South Africa pointed out that pressure to import GM crops is wreaking havoc on local economics in Africa. "In South Africa we are now dumping GM corn into other countries, disrupting local markets and undermining the livelihoods of family farmers there. As a result Zimbabwe has imposed a ban on GM corn imports. Kenya, which has a bumper crop of GM free corn and does not need any imports is grappling with a massive, illegal and unwanted shipment of 280,000 tons of GM corn from South Africa. A handful of powerful agribusinesses' obsession with GM is pitting African countries against each other, with Monsanto and international grain traders reaping the benefits and ordinary farmers losing out. The last thing we need from the US is a bill legislating yet more money for GM crops".

"At the end of the day, the GM mandate has more to do with breaking open markets for American biotech corporations than fighting hunger" – explained Annie Shattuck of the Institute for Food and Development Policy. To get at the root of the global hunger crisis, we need to tackle poverty, something no technological silver bullet can ever do". Ben Burkett, President of National Family Farm Coalition, and a Mississippi family farmer, added "corporate control over inputs and the free trade agenda have destroyed the livelihoods of so many farmers at home and abroad. That's why farmers worldwide are calling for food sovereignty – the right to choose fair and sustainable farming practices that protect our local food and livelihood security. This is what works best for our farms and communities".

Interlocking interests of big business, big philanthropy and US foreign aid : AGRA Watch was founded in 2008 to challenge the Gates Foundation's participation in Alliance for Green Revolution in Africa (AGRA), and to support instead, sustainable, agro-ecological alternatives already practiced in Africa. The Gates Foundation and the Rockefeller Foundations are partners in AGRA, and are also involved in numerous other projects aimed at spreading GMOs in Africa. According AGRA Watch, participants like Danforth Centre receives fund from the Gates Foundation as well as Monsanto Fund, the so-called philanthropic arms of the company for GMO promotion. The agenda of Biotech Corporation are clearly linked with each other and to US policy thereby gaining legitimacy and enormous influence over rest of the world. The US Global Food Security Act is clearly a desperate attempt to step up the aggressive agenda of pushing GMOs especially to Africa when GM crops are failing all over the world, particularly in the USA, the heartland of GMOs.

Major crops genetically modified for just two traits – herbicide tolerance and insect resistance – are ravaged by superweeds, farmers are fighting a losing battle with most sprays and increasing toxic chemicals. The failures, amazingly, are making the GM lobby more aggressive.

Monsanto's GM wheat shelved in the interest of US wheat export : In 2004 Monsanto declared that they would not cultivate the GM wheat they have developed commercially anywhere in the world for the time being. The reason that surfaced is the vitally important commercial issue of a possible collapse of American wheat exports. Robert Wisner, Professor of Economics in the Iowa State University and an international expert of global grain trade predicted that the release of GM wheat would reduce US export of wheat by half and cause a one-third reduction of wheat price. The document raised a furore amongst US and Canadian farmers, wheat exporters and millers that forced Monsanto to withdraw GM wheat for the time being. Prof. Wisner's second document in 2005 reiterating the inevitable financial loss to all parties (except Monsanto!) has more or less sealed the possibility of early release.

These issues are raised to emphasize that the same fate awaits Indian agricultural exports with the commercial release of genetically modified food crops in the country. The GM lobby (international and national) would like to see highly subsidized substandard GM food entering the country once our own GMs are commercialized. By the time genetic contamination as well as unethical market-driven propaganda will bring to all crops the same fate as with cotton whereby non-GM seeds along with their producers (both in the public and private sectors), as well as research institutes would become redundant. Perhaps in due course the whole public sector research establishment would have to play

the role of a second fiddle playing to the true of the over-dominating and powerful GM crop promotion machinery.

Labeling of GM products: A highly contentious but unavoidable issue in GMO product industry is the compulsory labeling of GM products especially for food. In the USA and Canada GM food products are not labeled on the basis that they are "substantially equivalent" to conventional products and need no segregation and labeling, while in the EU labeling is compulsory.

The term "substantial equivalence" has been a very cunningly used commercial-cum-political term (allegedly corroborated by President George Bush, Sr.) and made legal so as to avoid labeling of products. Scientifically, the very basis of GMOs i.e. the production of novel proteins (e.g., cry1 Ac toxin, about 16-17 mg per kg of Bt brinjal) by any stretch of scientific reasoning cannot be accepted as substantially equivalent to conventional brinjal. However, any elaboration or quantification of the term has been hotly contested by the GM lobby. Monsanto's recombinant bovine growth hormone (posilac) for boosting milk production in dairy cattle, however, faced a stiff challenge; growth hormone free milk labeling is defacto operative. It is definitely a just and genuine demand of consumers to know about the food they consume. Recent developments in different countries especially in the European Union and also in the USA itself indicate that the demand for labeling cannot be denied indefinitely.

Legalized labeling may, however, turn out to be the death-knell of GM food and its large scale commercialization.

Some Recent Positive Developments in the USA

Contrary to the long standing policy of the USA of promoting GMOs at home and abroad a number of recent developments need to be mentioned as those developments signify a growing anxiety and awkwardness because of powerful monopolistic corporate omnipresence and influence, affecting overall development and social harmony. These are as follows:

1. A reversal of the policy to allow large scale gene patenting: On November 11, 2010, US Government decided that gene patenting should not be granted for genomic DNA.
2. US government launches unprecedented anti-trust inquiry.
3. US Federal court orders first ever destruction of GMO crop, orders removal of genetically engineered sugar beet seed crop.

No patent for genomic DNA in USA : US Government dropped a bombshell on October 29, 2010 when it reversed a long standing policy stating that patents should not be granted for genomic DNA as it is a product of nature even when isolated from the organism. However, of recombinant DNA or new combination of DNA for making genetically modified organisms or gene therapy, or "Synthetic life remain patentable". The opinion was expressed by the US Justice Department in response to the ongoing battle over the breast and ovarian cancer predisposing genes BRCA 1 and BRCA 2.

As of 2010, approximately 40,000 US patents exists that relate to an estimated 2,000 human genes; patents have also been issued for isolated genes, methods of using isolated genes, and methods to diagnose a disease based on an association between a gene and a disease.

In any case, the ramifications of the decision of non-patentability of genomic DNA would be tremendous and signals the possible end of gene patenting – the basic foundation of the globalized commercial exploitation of natural DNA.

Anti-trust and regulatory enforcement : For the first time in history, the US Department of Agriculture (USDA) and the Department of justice have joined forces to organize a series of workshops from March to December 2010, to be held in different parts of the country that aims to "explore competition issues affecting the Agricultural sector in the 21st century and the appropriate role for anti-trust and regulatory enforcement in that industry".

Seed monopoly : For the first time in USA, one all-day public hearing, held in Ankeny, lowa was held on 12 March, 2010 which was attended by about 500 people, including farmers from several states, ranchers, company representative, local people and notably, representative from Monsanto, the company with near monopoly on seeds of maize and soybean. In the US, these seeds are mostly genetically engineered. Monsanto illustrates the breakneck speed of corporate concentration in the sector and presently controls 60% of corn seed market, 62% of soybean, 95% of transgenic cotton seed market and is rapidly consolidating control of vegetable, sugar beet and wheat markets. Monsanto's GE soybean and corn now cover 92% and 85% respectively of total US acreage of those two crops.

Issues raised in the hearing specifically included how the US farmers are being squeezed between high costs

of seeds and chemical inputs and low farm prices. They strongly expressed their grievances and opposition to the excessive control that "Big Ag" (specially Monsano) has gained, and pledged to fight back.

At the public hearing, US Attorney General Eric H. Holder Jr. conceded that "reckless deregulation has restricted competition in agriculture". Agriculture Secretary Tom Vilsack, also expressed concern over the present situation. "This is not just about farmers and ranchers. It is really about the survival of rural America". He said, "We have seen a significant decline in farmers and ranchers and that translates into a significant decline in the number of people living in rural America". Commenting on the joint involvement of the two governmental departments (USDA and Justice Dept.), Mr. Holder told reporters, "You will see an historic era of enforcement that will almost invariably grow from the partnership that we have established".

US Federal court orders first ever destruction of GMO crop, orders removal of genetically engineered sugar beet seed crop : On November 30, 2010 Federal Judge Jeffery S. White issued a preliminary injunction ordering the immediate destruction of hundreds of genetically engineered (GE) sugar beet plants planted in September, 2010 after finding the seedlings has been planted in violation of federal law. Judge White noted, "farmers and consumers likely suffer from cross contamination between GE sugar beets and non- GE sugar beets". He continued, "the legality of the Defendants' (USDA and Monsanto) conduct does not even appear to be a close question," nothing that the government and Monsanto try to circumvent his prior ruling, Which make the GT (glyphosphate tolerant) sugar beets illegal.

US courts have twice rescinded USDA's approval of biotech crops. The first such crop, Roundup Ready alfalfa, is also illegal to plant, based on the vacating of its deregulation in 2007 pending preparation of an EIS (Environment Impact Study). Although Monsanto appealed that case all the way to the Supreme Court and the High Court set aside part of the relief granted, the full prohibition on its planting – based on the same initial remedy granted here, the vacateur – remains in place.

Monsanto's RR crops presently cover 90% pf soy acres, & 70% of corn and cotton acres and 95% of sugar beet. Unfortunately, a Federal court has recently halted the future plantings of RR beets unless the USDA (that approved the same in 2008) completes an environmental impact study of their effects.

Roundup and soil health and productivity: a startling recent report : The endless repeated claim that Roundup Ready technology saves "millions of tons" of soil from erosion by allowing farmers to avoid tilling to kill weeds, appears to the widely trumped up. According to the Environment Working Group, the USDA's 2007 National Resource Inventory (NRI), " there has been no progress in reducing soil erosion in the corn belt since 1997", (it needs to be mentioned that the corn belt is the section of Mid-West where the great bulk of RR corn and soy are planted). EWG writes, "The NRI shows that an average-sized lowa farm loses 5 tons of high quality topsoil per acre each year". In short Monsanto's Roundup Ready technology is emerging as an environmental disaster. The question is not only why a judge demanded an environmental impact study of Roundup Ready sugar beets in 2010, it is that why no one asked that before the technology was rolled out in 1996. It needs to be reminded that the Union of Concerned Scientists raised the issue at that time but seed developers did not pay any heed to that. The USDA always supported the seed developers.

In a better world farmers would be looking to non-chemical methods for controlling weeds: crop rotations, mulching, cover crops, etc. Instead they are being paid by Monsanto to rampant application of poisons.

Despite the overall detrimental effects of Bt cotton, why is this mad rush for Bt cotton in India?

The presently cultivated Bt cotton in India has failed in many of the promises by the seed developers; there has not been any increase either in intrinsic or in operational yield, also no significant reduction in chemical insecticide sprays. The insects like pink bollworm (*lalia*) has developed resistance and the parent company Monsanto has publicly admitted that failure and has suggested acceptance of an improved GM version for the purpose. The GM crops are more sensitive to weather extremes, Bt toxins exuded by roots and released by decomposition are injurious to soil health and invite pathogens (Sarkar et al., 2008) and above all it has successfully displaced (rather wiped out the non-GM cotton cultivars for many of which seeds for planting are unavailable – cotton crop diversity of the country is at stake; for obvious reasons no varieties are now being released by CICR, Nagpur). Planning Commission experts recommended that Bt cotton should not be cultivated in arid rainfed areas but that continues unabated. There is no doubt whatsoever that farmer suicides have been greatly exacerbated by Bt cotton cultivation. Death of livestock, cattle, sheep, goat, etc.

has been so widespread that concerned state government officials in A.P. had to officially warn against the traditional practice of grazing on cotton wastes. The question why is this mad rush for a technology that should have been rejected in the first place.

Professor G. D. Stone (Department of Anthropology and Environmental Studies in the Washington State University, USA) in a survey based informative research paper in 2007 on Bt cotton cultivation in Warangal district of A.P. analyzed the reasons for the large scale switch over and attributed the same to deskilling of farmers specially loss of traditional knowledge passed on from forefathers to subsequent generations and tremendous localized fad amongst farmers generated due to false and unreasonable propaganda by seed developers, dealers, retailers as well as influential people. Being mesmerized by exceptional future prospects they went for the seeds. Eventual inadequacies and failures were accepted by these poor and simple farmers as personal lapses.

GM crops which are all F_1 hybrids (including those in the pipeline) and cannot be saved for sowing by farmers that would take away not only food security but also food sovereignty which is most condemnable. Seed Bill, 2004 lying before the Parliament needs serious rethinking and redrafting to ensure categorically the legal rights of farmers to save all seeds produced by them.

Global Climate Change and Agricultural Systems

Agriculture along with deforestation and changed land use collectively account for the highest anthroprogenic GHG emission of 30.9% of total emission and as such, with maximum global warming potential (IAASTD, 2008). Globally, total anthropogenic GHG emission in terms of CO_2–eq. in 1970 was 28.7 gigatons per year while in 2004 the value went up by 70% to 49.0 gigatons per year (IPCC, AR 4, 2007). The situation is really serious and immediate adaptation to changed climate scenarios as well effective GHG mitigation strategies must be worked out by agricultural scientists. Countries in the low latitudes (mainly developing countries) would suffer more due to global temperature rise in comparison to those in the high latitudes (mostly developed countries) with greater resources to tackle climate change eventualities.

The most effective strategy to adapt to global climatic changes particularly to high temperature, unpredictable rainfall pattern and weather extremes would be to put due emphasis on increased biodiversity. Judicious use of different crops as well as different varieties within a crop species for cultivation in the same field or as mixed stand in alternate lines or cluster of lines (or by broadcasting seed mixtures) would serve as natural insurance against climate change induced biotic and abiotic stresses.

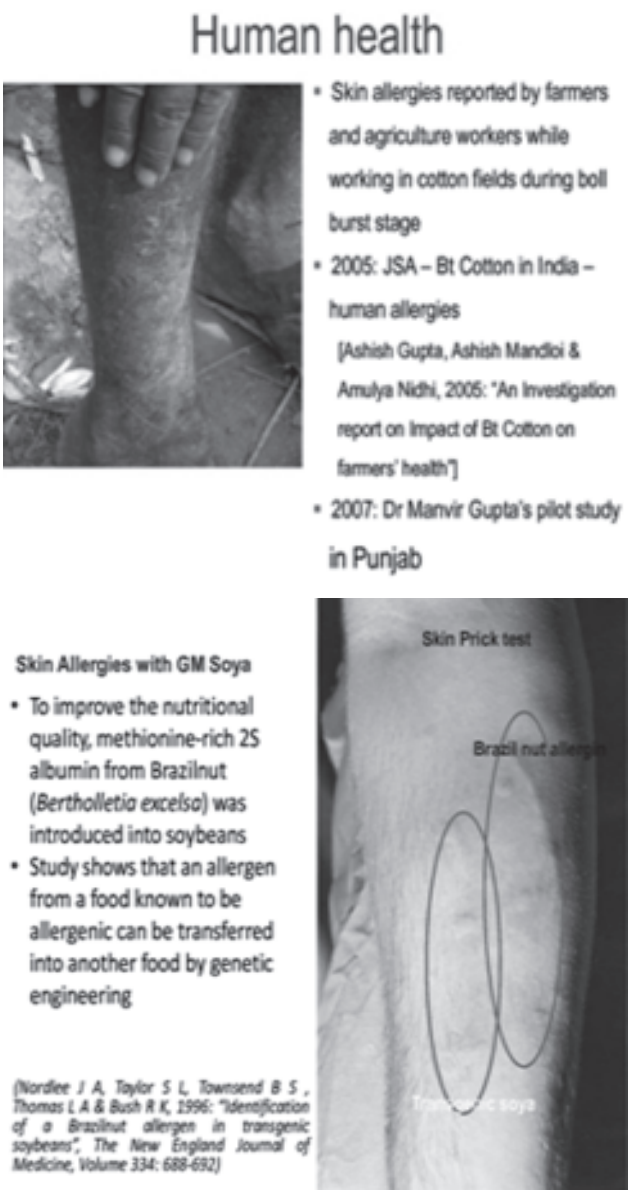
Inclusion of drought tolerant and water logging resistant varieties in the same or adjacent crop stands may enable farmers to tide over uncertain climatic conditions particularly rainfall patterns. Instead of GM crop which be unsuitable for irregular temperature and rainfall patterns, breeding of varieties through marker assisted breeding (MAB) and marker assisted selection (MAS) has been advocated by IAASTD and also ecologically sustainable natural resource management and biodiversity based agricultural systems have been suggested by IAASTD (2008) particularly for the small holder farmers of the world.

The story of Hiware Bazar, a village with 54 millionaries in drought-prone Ahmednagar district in Maharashtra is really an inspiring example of integration of livestock farming, grass growing and selected crops through comprehensive microwatershed management (Hiware Bazar Calling : Cover story, Down to Earth, January, 31, 2008 pp 28-31).

GHG emission and energy saving by organic agriculture : The nearly 31% contribution by agriculture and related land use changes including deforestation to total anthropogenic GHG emission (IAATD, 2008) could be very effectively mitigated by organic agriculture systems along with over 17% saving of fossil-fuel energy as compared to conventional agriculture, through phasing out synthetic nitrogen fertilizers (Mae-Wan Ho, 2008), which besides ensuring food security and sovereignty would result in effective mitigation of the adverse effects of climate change.

Health Issues Involving GM Crops

As early as 1996, Nordlee and coresearchers identified a Brazilnut allergen in transgenic soybeans genetically modified to improve the nutritional quality through introduction of gene coding for methionine rich 2S albumin from Brazilnut that being the first published evidence to show that an allergen can be transferred (of course unintentionally) into another food source by genetic engineering. Subsequently, TerjeTraavik and Jeffrey Smith (2004) provided evidences of allergic reactions in Filipinos exposed to pollens from Bt cornfields showing skin allergies, respiratory problems etc. with blood tests showing an immune response.

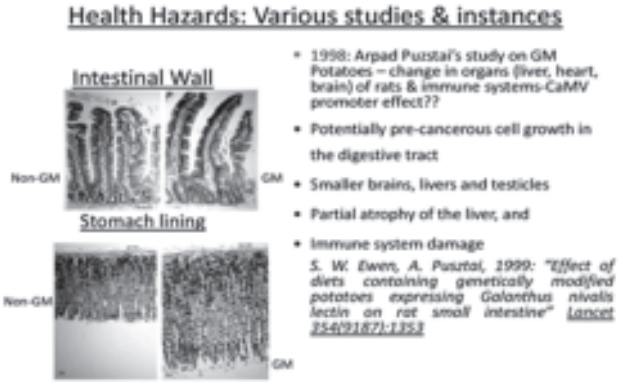


Skin contact with Bt toxin would also trigger allergic reactions. In a medical report by a team of doctors in 2005, hundreds of farmworkers in Madhya Pradesh in India were found to develop allergic symptoms when exposed to Bt cotton but not in those exposed to non-Bt varieties.

Reputed toxicologist Arpad Pusztai who led a team of researchers on a rat feeding experiment with genetically modified potatoes made some comments to a television interviewer in 1998 on the adverse effects on rats and expressed his doubts on the sustainability of GM potatoes as a human food; the same was broadcast as a news item by a major channel and created a huge uproar amongst British consumers. Dr. Pusztai was ordered by the authorities not to speak on the issue and he along with his wife Dr. Susan Bardocj, a coresearcher, were ultimately dismissed from service. The damage was, however, done and the growing public protest against GM food forced the British Government to declare a moratorium on GM food. Other European countries did the same .The episode greatly perturbed the powerful GM lobby (that besides corporate interests also included influential scientists of even the Royal Society, London).

In the meantime the editor of the internationally famous British medical journal Lancet published two papers of Pusztai and another colleague Dr. Ewen in which swellings of stomach walls and intestinal linings of GM- potato fed rats were clearly shown. Even the editor of Lancet was not spared for publishing the same and was bitterly criticized by the proponents of GM technology. But the critics did not take any step to verify the truth and in due course of time the findings of Arpad and coworkers were confirmed by many independent researchers.

Arpad Pusztai

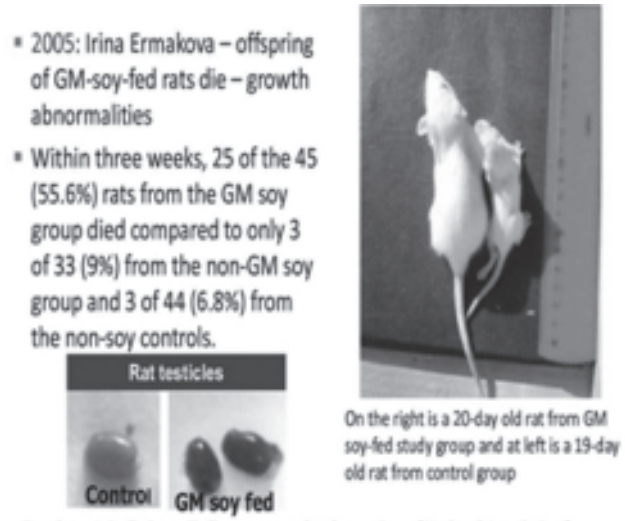


In 1998, The Russian Academy of Medical Sciences recorded higher organ and tissue damage in GM potatoes (resistant to colorado beetle) and categorically declared the same as "not safe to be used in the nourishment of people." Interestingly, the work was carried out under agreement with the Monsanto Company and the full report of 275 pages, and raw data have been provided.

In 1999, Marc Lappe and coresearchers published a paper in the *Journal* of *Medicinal Food* (4; 241-45) showing lower levels of beneficial phytoestrogen compounds in genetically modified herbicide tolerant soybeans. It is worthwhile to recall in this connection the research work of Stephen Padgette and his colleagues (1996) who reported twice the content of soy lectin which

can block nutrient assimilation in cooked genetically modified (glyphosphate tolerant) soybean seeds.

Malatesta *et al*. (2002) studied the effect of feeding GM (glyphosphate tolerant) soybean on mice and noted significant changes in the nuclei (irregularly shaped nuclei) in the hepatocytes of the liver of GM- fed mice.

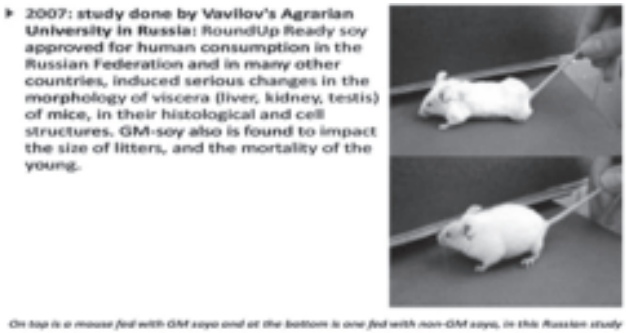


Malatesta *et al*. 2003 also studied the effect of feeding GM soybean on the pancreatic acinar cell nuclei of mice. The modifications in the acinar cell nuclei could be related to the reduction in digestive enzyme synthesis and secretion which would influence pancreatic metabolism in mouse.

Irina Ermakova (2005) of Russia presented her findings on the influence of genetically modified soya on the birth weight and survival of rat pups at the Symposium of National Association for Genetic Security on October 10, 2005. She recorded growth abnormalities and premature death of GM soyabean fed rats. Within three weeks,25 of the 45 rats (55.6%) from the GM soy group died compared to only 3 out of 33(9%)from the non GM soy group and 3 out of 44 (6.8%) from the non-soy controls. There was a significant reduction in the size of testicles of GM- soy fed rats.

Irina Ermakova

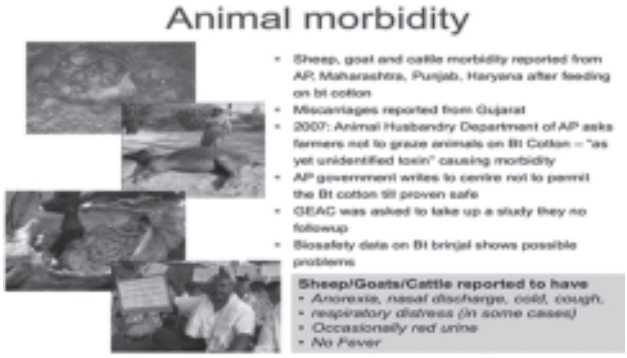
A study conducted by Vavilov Agrarian University in Russia in 2007 showed that a genetically modified Roundup Ready soybean, approved for human consumption in the Russian Federation and in many other countries, induced serious changes in the morphology of liver, kidney, testis as well the respective histological structures of mice fed with the GM- soy. The size of litters as well as mortality of the young was also affected thereby reconfirming Ermakova's earlier observations.



Animal Morbidity in the Bt cotton fields in India

Grazing of sheep, goat and cattle on cotton fields has been a traditional practice in Andhra Pradesh, Maharashtra, Madhya Pradesh and other cotton growing states in India. But after switch over to Bt cottons reports on animal morbidity started to pour in at regular intervals following grazing on leaves and left over materials of Bt cottons. Miscarriages were reported from Gujarat.

In 2007 the Animal Husbandry Department of Andhra Pradesh advised the farmers not to graze animals on Bt cotton fields because of "as yet unidentified toxin" causing morbidity. The State Government being concerned with the issue requested the Centre not to permit Bt cotton cultivation till proven to be safe. The Genetic Engineering Appraisal Committee (GEAC) was requested to take up a study on animal morbidity but there was no critical follow up.



Animal morbidity was associated with anorexia, nasal discharge, cold, cough and in some cases acute respiratory distress.

A long term feeding study commissioned by the Austrian Agency for Health and Food Safety (under the Austrian Federal Ministry of Health) was carried out by the Veterinary University, Vienna confirmed that genetically modified corn seriously affected reproductive health in mice. Professor Zentek of the Veterinary University, the reputed lead author , reported a direct link between the decrease in fertility and the GM diet; mice fed with non-GM corn reproduced more efficiently (incidentally the immediate fall out of the Austrian Government report was a substantial fall of Monsanto's share price).

At around the same time Italian researchers Finamore *et al*. reported intestinal and peripheral immune response to insect resistant (Bt) MON 810 maize ingestion in weaning and old mice.

The aforesaid Bt corn MON 810, as well as two others (NK 603 and MON 863) were used for a comparative study by de Vendomois *et al.* (2009) as regards food safety of the three commercial GM maize types. All of them caused hepatorenal toxicity of rats used in the feeding experiment, adverse effects were also noticed in the heart, adrenal glands and hematopoietic system.

In a critical review of Monsanto- Mahyco's biosafety data on Bt brinjal in India, as commissioned by Greenpeace, Dr. Gilles-Eric Seralini of the University of Caen and President of the Scientific Council of the Committee for Research and Information on Genetic Engineering (CRIIGEN) pointed out the methodical inadequacies in the study design and concluded that the interpretation of the results was not scientifically acceptable. As such, consumption of Bt brinjal cannot be considered safe (Seralini, 2009). Similar views were expressed by Dr. Judy Carman of the Institute of Health and Environmental Research (IHER) in January, 2009.

Professor Jack A. Heinemannn, Director of the Centre for Integrated Research in Biosafety, New Zealand and an international authority in the subject sent a detailed highly technical report in July 2009 stating categorically that the safety concerns had not been addressed. According to Dr. Heinemann, "The long accepted version of cry toxicity is not the actual mechanism. Thus the range of organisms that will find cry toxic may not be predicted from knowledge based on toxicity screening of the cry proteins alone."

In this connection a meta-analysis of data by Seralini *et al.* 2011 on previously carried out 19 different GMO feeding experiments with new biological and statistical methods showed differences related to the sex of the test animals that would be worth mentioning. For example, 43% of significant abnormalities were found in the kidneys of male animals. The liver was more affected in the females, opening up a new dimension to safety concerns.

Aris A, Leblance S. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reprod Toxicol (2011), doi: 10.1016/j.reprotox.2011.02.004 :

An outstanding evidence of GMC-associated toxins crossing over the human placental barrier has been provided in the first ever study with pregnant women in Quebec, Canada by Aris and Leblanc in 2011. Maternal and foetal exposures to pesticides associated with GM foods such as the herbicides glyphosate and glufosinate or insecticidal bacterial toxins (Bt) were studied in 30 pregnant women, keeping 29 non-pregnant, otherwise of equivalent physical condition, for comparison. A toxic metabolite of the herbicide glufosinate (3-methyl phosphinico propionic acid, MPPA) and the Bt toxin, cry1 Ab, were detected in the pregnant women, their fetuses and also in the non-pregnant women. The toxins which were not eliminated would, as such, be a serious threat towards the development of the foetus. The matter must be treated with utmost seriousness as the well-being of our newer generations is at stake. Certainly the time is ripe enough for a total ban on the commercial use GMOs and associated pesticides in agriculture to save mankind from eventual inevitable disaster.

In connection with health issues the horizontal gene transfer (HGT) involving antibiotic resistant marker gene as used in many Bt crops may lead to undesirable consequences with intestinal microbes picking up the antibiotic resistance gene.There is such a possibility in case of the antibiotic kanamycin which is currently used in many infectious diseases and is a second line of

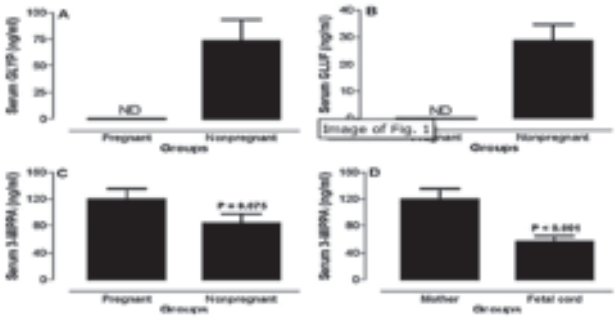


Fig. 1. Circulating concentrations of Glyphosate (GLYP: A Gluphosinate (GLUF: B) and 3-methylphosphinicopropionic acid (3-MIPA: C and D) in pregnant and nonpregnant women (A-C) and in material and fetal cord blood (D). Blood sampling was performed from thirty pregnant women and thirty-nine nonpregnant women. Chemicals were assessed using GC-MS. *P* values were determined by Mann-Whitney test in the comparison of pregnant women to nonpregnant women (A-C). *P* values were determined by Wilcoxon matched pairs test in the comparison of material to fetal samples (D). A *P* value of 0.05 was considered as significant.

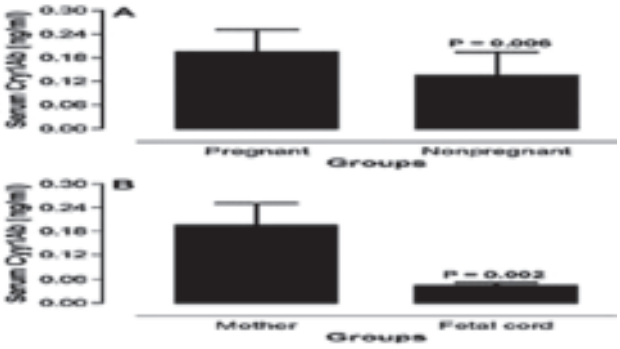


Fig. 2. Circulating concentrations of Cryl Ab toxin in pregnant and nonpregnant women (A) and material and fetal cord (B). Blood sampling was performed from thirty pregnant women and thirty-nine nonpregnant women. Levels of Cryl Ab toxin were assessed using an ELISA method. *P* values were determined by Mann-Whitney test in the comparison of pregnant women to nonpregnant women (A). *P* values were determined by Wilcoxon matched pairs test in the comparison of material to fetal samples (B). A *P* value of 0.05 was considered as significant.

treatment for tuberculosis (TB). Drug resistance TB is now a serious threat to India and it would be a matter of anxiety if an important second line drug is lost (Singh, 2009). Dr G.P.I Singh, himself a reputed physician is rightly concerned with the issue, though currently antibiotic resistant marker genes are not used in view of the aforesaid consequences, the question is about all those marker genes already in circulation that cannot simply be recalled.

Professor Don Huber on Genetically Modified Glyphosate Resistant Crops

Dr. Don Huber, a reputed retired scientist, now an emeritus professor at the Purdue University, USA wrote on January 16, 2011 a personal letter to the US Secretary of Agriculture, Tom Vilsack requesting him not to permit commercial release of the herbicide glyphosate (trade name Round Up) resistant GM alfalfa , an important fodder crop as that would very adversely affect livestock health. The content of the letter were explosive in that the world's topmost herbicide glyphosate (Round Up) itself was held responsible for the stimulation of a virus like pathogen that would act as a super pathogen because of its virulence.

According to Huber, the greatly increased use of glyphosate (Round Up) in such crops as RR soy, RR corn etc. has provoked the generation of the super pathogen. Farm animals, that are fed GM crops grown in glyphosate treated fields have shown an alarming increase in sterility, spontaneous abortions and still-births. GMO feed grown on glyphosate treated fields tends to irritate the stomach of livestocks, such that many farm animals are fed daily rations of bicarbonate of soda in an attempt to sooth their stomach linings.

Serious concern is raised about glyphosate's multifarious effects (it was originally patented by Stauffer Chemical Company in 1964 as a metal ion chelating agent; the patent by Monsanto as a herbicide was subsequent to that in 1974, it has also been patented as an antibiotic besides use as a ripening agent). About 40 plant diseases such as chlorosis, sudden death syndrome (SDS) of soybean, Goss' wilt in corn, etc. have been linked with glyphosate. Also a host of human diseases (as direct or indirect consumers of glyphosate treated crops, especially genetically modified RR crops requiring exclusive use of glyphosate) appears to be associated with the situation that include (i) increase in cancers of the liver, thyroid, kidneys, testes and skin melanomas, (ii) increase in allergic reactions (up to 50% increase in soybean allergies in the USA in the last three decades), (iii) over 90 times increase in Alzheimer's disease in the same period and projected to be much faster in the coming years, (iv) increase in the rate of Parkinson's disease which researchers believe is significantly provoked by toxic pesticides.

All the aforesaid observations and issues raised by Dr. Don Huber are indeed of great concern and deserve utmost consideration of policy-makers dealing with vital issues on food and agriculture, especially those specifically involving genetically modified crops and associated pesticides.

It was no wonder that the American Academy of Environmental Medicine after reviewing the literature came to the conclusion that the GM foods which have not been properly tested for human consumption could be unsafe and there is ample evidence of probable harm. The Executive Committee of the Academy recommends the public to avoid GM foods when possible and asked the members to provide educational materials concerning GM foods and health risks. It has also asked for a moratorium on GM food and implementation of immediate long term independent safety testing and labelling of GM foods which is necessary for the health and safety of consumers.

We may recall in this connection that the British Medical Association, UK after the famous toxicologist Arpad Pusztai's revelations of the adverse effect of GM food, demanded a total ban on the GM food for human consumption. That stand by the largest body of its kind in Britain had a great effect on the public in general not only in Great Britain but also all over Europe.

Don Huber's letter to the US Secretary of State for Agriculture on the pathogen stimulating effect of glyphosate would also greatly rekindle the interest on

earlier works on the subject by other researchers. Robert Kremer and his colleagues in the agricultural research service of the United States Department of Agriculture (USDA) noted a clear involvement of the *Fusarium* fungus induced pathogenicity with glyphosate; the active ingredient of the Roundup herbicide. Roundup Ready (RR) soybean plant grown in sterilized soil would show a little stunting of plant growth in presence of glyphosate but in normal (non-sterilized) soil, the plants were killed in presence of glyphosate. In the control (without glyphosate), RR soybean plants grew normally, suggesting involvement of pathogenicity (normal non-sterile soil) and glyphosate. Studies on fungal colonization with root sections of RR soybean plants clearly corroborated the induction of pathogenicity by soil borne *Fusarium* fungus (Kremer and Means, 2009).

In a paper in 2006, Eker *et al.* and co-workers (Eker *et al*. 2006, *J. Agric. Food Chem*) recorded reduced root uptake and transport within the plant of iron, manganese and zinc in the presence of glyphosate, the most affected being manganese (uptake only 20% of control, transport 2% of control). We should remember that glyphosate was originally patented as a metal chelating agent, as such the aforesaid findings are only to be expected and (with unavailability or inadequate availability of essential mineral elements,various plant deficiency diseases would occur (farm animals and human beings as direct or indirect consumers would suffer from a host of deficiency diseases as a fall-out of that).

Robert Kremer rightly observed that they as researchers were setting up a big problem and expressed alarm that the regulators were not paying enough attention to the potential risk from biotechnology on the farm. The on-farm scenario is indeed extremely complex and getting all the more complicated with time (see Jeffrey Smith, 2011 for further obsrvations on the issues by Don Huber, Robert Kremer and others).

Soil Health Considerations: Soil health is of vital importance for successful sustainable agriculture irrespective of other considerations. As such, maintenance and if possible improvement of soil health, should be basic to all farming systems and technologies.

The endless claim by Monsanto that its Round Up Ready Technology saves millions of tons of soil from erosion by allowing farmers to avoid tilling to kill weeds, appears to be widely trumped up on. According to the Environment Working Group, the USDA 's 2007 National Resource Inventory (NRI) , there has been no progress in reducing soil erosion in the corn belt since 1997. We have already discussed how glyphosate (Round Up) and Round Up Ready (RR) GM crops stimulate soil pathogens and provoke diseases (Kremer and Means 2009) and adversely affect uptake and transport of mineral elements within the crop plant (Eker *et al.* 2006).

According to Johal and Huber(2009), the effects of glyphosate on plant diseases could be the results of direct glyphosate induced weakening of plant defences and increased pathogen population and virulence. Indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigour of plant from accumulation of glyphosate in meristematic root shoot and reproductive tissues, altered physiological efficiency, or modification of soil microflora affecting the availability of nutrients involved in physiological disease resistance.

Yield reduction of crops grown in rotation following harvest of GM Round up Ready crops receiving high dose of glyphosate (Round up) has been widely reported by farmers in the USA because of the residual toxicity in the soil. According to Huber (2011), immobilized glyphosate can be reactivated in soil and be a serious problem for other crops in rotation.

Farmers in different states of India who have switched over to Bt cotton as in Andhra Pradesh, Karnataka, Punjab etc. are reporting yield decreases of crops grown after Bt cotton. It is possible that the Bt toxins in the roots are somehow adversely affecting the soil quality and fertility. An important research paper published in 2008 by researchers in the Indian Agricultural Research Institute (IARI), New Delhi (Sarkar *et al*. 2008) confirmed that transgenic Bt cotton affects enzyme activity and nutrient availability in subtropical inceptisol, a class of soil that nurtured the crop. It is imperative that the issue must be taken up immediately by decision makers to save the already economically hard pressed farmers forced by prevailing circumstances to cultivate Bt cotton. (the public sector institutions as well as private companies have virtually no non-Bt cotton seeds for sale to the farmers of the country!)

Coexistence With GMCs Not Possible

Contamination is inevitable; whenever and wherever GMCs have entered they have contaminated traditional, organic and conventional varieties and landraces of cross pollinated plants, in particular, with considerable rapidity.

Genetically modified genes spread to local maize in Mexico : To protect the native /germplasm of maize (corn) of Mexico, a centre of origin and of great biodiversity, the Mexican government in 1998 outlawed the planting of GM maize to protect approximately 60

domesticated landraces and their wild relatives. However, as per newspaper reports around 70 hectares were clandestinely sown with GM maize from across the border, the USA. In 2001, David Quist and Ignacio Chapela provided the first evidence of transgenics getting into landraces in the reputed science journal Nature that created a huge uproar and controversy as the powerful GM lobby refused to accept the findings on flimsy technical grounds (that is usual practice!) but Quist and Chapela firmly stood by their conclusions. Being influenced by the political pressure, Nature in 2002 published an additional note saying there was insufficient evidence to justify the original publication that pacified the advocates of GM crops who widely and erroneously called this a retraction. In 2008 Elena Alvarej- Buylla and others of the National Autonomous University of Mexico (UNAM) confirmed the entry of transgenes into traditional maize crops. According to Rex Dalton who has made a prepublication review of the paper in Nature (full paper published in the journal, Molecular Biology) has narrated how difficult it has become to work neutrally in the highly vitiated political atmosphere. Nature has, however, recovered some of its lost prestige on the issue by coming out with the rejoinder noting in particular, "The importance of the study is not the impact of the transgenes themselves but the fact that their spread has occurred so easily."

Mahyco's GM rice contaminates natural rice in Jharkhand, India : Jharkhand, (along with Orissa and Chattisgarh) is a centre of origin of rice, the region where the maximum genetic diversity of rice is found.

GM rice carrying Bt gene was planted by the Mahyco Company (as Indian joint venture with Monsanto) in field trials in Saparong village in Ratu block, Ranchi. violating all rules for such trials. Dr. Suman Sahay, convener of Gene Campaign with her staffers collected seeds and leaves from the second generation rice plants (as well as unburnt rice plants that had been thrown on the bunds) and sent samples for testing in the laboratories of EurofinsGeneScan in Germany (a highly reputed testing facility and a world leader in testing GM crops). In January 2009, Dr. Sahay presented the reports which confirmed that that the seeds and leaves were contaminated with Cry 1 Ac gene that was originally used in the Bt rice being developed by Mahyco. As such, the test results provide conclusive evidence that the Bt gene was allowed by Mahyco to escape into the environment. This is all the more important and of great environmental concern in an area where genetic diversity of rice is found and where damage to the rice gene pool could be maximal. Such development would certainly jeopardize India's rice exports to markets in Middle East, Europe etc. Dr. Sahay very appropriately urged the GEAC to take further action to seek damages from Mahyco as per provisions of the Environment Protection Act, 1986.

The experience of some of the present authors (TKB & RNB) as members of the West Bengal State Agriculture Commission (2007-2009) on the shoddy field trials on GM rice in West Bengal has been equally disappointing. The commission strongly recomended a ban on GM crop field trials in West Bengal.

A few years ago in USA , GM rice from a single field trial conducted by Ventria Company found its way to US rice exports and caused a havoc; and USA had to recall contaminated rice from different countries costing millions of dollars. The event led to the crash of America's rice export markets causing huge long term losses to American rice growers.

According to Percy Schemeiser, a famous anti-GM vocalist of Canada, GM contamination has totally destroyed the European Market for soybean and canola (rapeseed) of Canadian organic farmers. "All our seed is contaminated. We can no longer sell one bushel of canola to the European Union. We have lost our market all over the world", Schmeiser laments.

Everywhere a handful of GMCs have thrown out of cultivation (maybe for good!!) a very large number of non GM biodiverse edaphoclimatologically well adapted local varieties.

SUMMARY & CONCLUSIONS

An appraisal of the global scenario would reveal a bleak future of GMCs everywhere including the heartland of the GMOs, the USA. Thousands and thousands of acres of cotton are abandoned due to serious attacks by tarnished plant bug (TPB), *Lygus lineolaris* along with a range of resistant insects; palmer pig weed, *Amaranthus palmeri* along with over a dozen of super weeds enjoying extra large doses of glyphosate (even several other herbicides including 2,4-Dichlorophenoxy acetic acid, a constituent of Vietnam war-famous Orange G) defying the operation of combine-harvesters, one of the backbones of modern monocultural agriculture; soil ruined by Bt toxins released from Bt crops via life-long switched-on long-lived biopesticide production machinery; the broad spectrum metal chelating agent glyphosate [N-(phosphonomethyl) glycine] residues such as AMPA (aminomethylphosphonic acid), along with Roundup's so-called 'inert ingredients'(such as

polyethoxylated tallow amine, etc.), ruining the soil health and root system of RR soys suffering from of deficiencies of a range of elements like Mn (without Mn, photolysis of water – a key step in photosynthesis and many other enzymatic activities would be inoperative); with increased fungal diseases (always associated with morbidity and degeneration), and many more thing going to be disclosed soon. All these have already given high-sounding wake up calls in the USA with the Justice Department reversing the current practice of granting genomic DNA patents (with relief to GMOs – may be temporarily!); anti-trust moves against big Agri-corporate sector (with public hearings dominated by protesters belonging to family-farmer groups against the present situation); federal courts ordering the first ever uprooting of GM sugar beet plants already planted (with USDA's approval), the GMC technology is certainly facing meltdown in its home turf.

EU has no place for GM; Latin America offers little scope for further expansion. Hence the battle for extension to other parts of the world continues. Early commercialization of Monsanto's GM wheat, still in cold storage since 2004, is difficult due to internal resistances. The battle in Africa has been taken up with the help of mega-philanthropies like the Rockefeller Foundation, Bill and Melinda Gates Foundation, trying to push the GMO agenda influencing AGRA (Alliance for Green Revolution in Africa) for the purpose, and in the same way in our country, via the Indo-US Knowledge Initiative in Agriculture (KIA), and influencing people with power in and outside the central and state governments in India. South African GM corn produced through similar means have no market and a ship carrying 280,000 tons of GM remained unloaded for months together in Mombasa as Kenya refused to accept the same. A similar fate awaits us if India ultimately falls in the trap.

Tinkering with nature is proving dangerous. It does not require a high level of scientific knowledge to understand that organisms with short life spans will circumvent the problem through continued exposure via various resistance / tolerance imparting gene transfer mechanisms including mutations and further generation of antibiotics, etc. churned out by the world's multibillion dollar pharmaceutical industries virtually minting money while fighting against the never ending menace of development of antibiotic resistance of disease causing microorganisms but highly remunerative for seed developers to keep short-lived patents on GM events, cumulatively everlasting, a truly lucrative future indeed!

The proponents of GM technology argue that GM crops are the answer to the hunger of millions of poor people of the world which, however, is a myth. Firstly, hunger is not due to lack of food in the market but to lack of money to buy plenty of food available. Very limited access to credit for agricultural inputs to grow enough food is a major reason for the failure of the farmer to feed his family and sell the surplus to other consumers.

Secondly, the vast majority of GM crops cater to the provision of animal feed for the ever growing livestock industry and serve as raw materials for biofuels on a large scale; growing GM cotton for textile mills has almost turned non-GM cotton a rare commodity in many countries.

Thirdly, it has been admitted that with most GM crops there is little yield advantage, in fact except in GM maize where there is a minor yield gain (partly attributed to heterosis effect), for most other crops these is no yield gain; in soybean, there is a distinct loss in yield which even the USDA has admitted.

Fourthly, official data from the major GM crop producing countries would categorically show that the use of pesticides is rising and those include toxic chemicals banned in many EU countries. Besides rise in expenditure, there are increasing environmental and health problems of poor communities living near the intensively cultivated GM farms.

Lastly, the real beneficiaries of the GM model of farming are the companies who are making huge profits with their patented seeds and associated (often legally tagged) expensive pesticides and other agrichemicals. Of course the profit would percolate down to the general shareholders of the company but that is a part of the usual meticulously drawn out plan of the corporates.

But then why, inspite of aforesaid limitations are the farmers continue to grow GM crops, ought to be analysed. It appears that the labour-saving effect of the GM crop labour management system has been the main reason. The rapid spread of the GM herbicide tolerant soybean in Argentina (glyphosate tolerant soybean would permit large overdosages of the herbicide without harming the crop). According to a top-ranking official of the Argentine agriculture department, the significant labour saving effect means that only one new job is created for every 1,235 acres of land put under soybeans. The same amount of land devoted to conventional food crops on moderate size family farms would support four to five families and employ at least half-a-dozen people. For corporate export-oriented farming with a purely commercial motivation, glyphosate-tolerant soybean requiring just one job creation would be the first choice.

Agroecological and environmental issues would naturally be shelved, at least for the time being!

GMOs or GMCs in agriculture cannot and must not be viewed in isolation. The issue is really global. It is commendable but incidental that the Indian Parliament has been looking into the subject and a high-level committee deliberating on the issue of GM crops in India has called for a moratorium on GM-food crops. Perhaps many more countries will do the same.

A technology that is destined to fail must not be accepted, more so when the disaster would be irreversible and future generations would surely blame us for this misadventure on our part. The damage is not merely a possibility but an absolute certainty; and the frequent weather extremes, drought followed by untimely heavy rainfall would pay havoc with tailor-made GM crops having least sensitivity to weather extremes. GM crops must not be promoted at any cost.

Natural resource management based ecologically sustainable, wherever possible crop-livestock integrated, organic or near organic agriculture systems managed by the farm communities themselves, would be the key to food security and sovereignty besides effectively mitigating the adverse effects in global climate change. Such an approach would necessitate a total ban on genetically modified crops in India.

REFERENCES

Aris, A. and Leblanc, S. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive Toxicology, 31528-31 Elsevier, Journal home page :www.elsevier.com/ locate/ reprotox.

Basu, R.N. 2007. Genetically modified crops (GMCs): Special Guest Editorial. Indian Biologist, **39**(1), 89-96.

Basu, R.N.2007. The rationale behind an immediate return to crop-livestock integrated organic agriculture by small and marginal farmers. Proceedings of the National Workshop on "Organic Horticulture – It's Production,Processing,Marketing and Export for Sustainability", June 8-10,2007 (Chattopadhay, P.K. and Mitra, S.K. eds), BCKV, West Bengal, pp 16-35.

Basu, R.N. 2008. Indian agriculture at the cross roads : An imperative need to turn to crop-livestock integrated sustainable organic farming by small holders. Indian Agriculturist, 50 (Golden Jubilee Number), pp. 32-49.

Dalton, R. 2008 Modified genes spread to local maize: findings reignite debate over genetically modified crops. Nature, 456,149.

Ermakova, Irina 2006 Proceedings of the Conference on Epigenetics, Transgenic Plants & Risk Assessments, Institute for Applied Ecology, Frankfurt, 2006, pp 41-48.

Ewen, S.W.B. and Pusztai, A.1999. Effects of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine, Lancet, **354**, 1353-4.

Finamore *et al*.2008. *Journal of Agricultural Food Chemistry*, **56** (23), 11533-9.

Gene campaign http:/www. genecampaign.org

Gupta, A. *et al.* 2005.Impact of Bt. cotton on farmers' health in Barnani and Dhar districts of Madhya Pradesh. Investigation report Oct-Dec, 2005.

Huber, D.M., 2011. GMOs, Glyphosate and Tomorrow: An Interview with ACRES, USA; Published in Vol 41, No 5 of Acres, USA, The National Journal of Sustainable Agriculture, May 2011. Reprint pp.1-9.

Huber, D.M. 2011. Letter of reputed retired scientist, Dr. Don Huber to the US Secretary of Agriculture Tom Vilsack on the adverse consequences of Monsanto's Round up herbicide and Roundup Ready GM crops; letter written on January 16, 2011 (available on most GM-related websites).

Also see Don Huber on GMOs, Glyphosate and Tomorrow: An Interview with ACRES,USA: Published in Vol 41, No 5 of Acres, USA, The National Journal of Sustainable Agriculture, May 2011. Reprint pp.1-9.

Intergovernmental Panel on Climate Change (IPCC). 2007. Fourth Assessment Report, available at http://ipcc.ch.

International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD). 2008. Synthesis Report. Available at http:// www.agassessment.org

Institute of Science in Society (ISIS), 2008. Which Renewables? ISIS Press Release, 30/07/08. Available at http//www/i-isis.org.uk/index.php.

Institute of Science in Society (ISIS), UK. 2008. Press release (Mae-Wan Ho, October 10). Organic and localised food and energy system for mitigating climate change. Available at http:// www/ i-iss.org.uk/index.php.

Institute of Science in Society (ISIS), UK 2012. ISIS Report on Don Huber's presentation on UK Parliament by Eva Sirinathsinghji on 09/01/12. USDA Scientist Reveals All: Glyphosphate hazards on crops, soils and consumers. Available at http://www/i-iss.org.uk/ index.php.

ISIS Report, 2010. Glyphosate Tolerant Crops Bring Diseases and Death. Link: http://www.i-sis.org.uk/ glyphosateTolerantCrops.php

Johal, G.S. and Huber, D.M., 2009. European Journal of Agronomy **31**, 2009.

Malatesta *et al.* 2002. Cell Structure and Function **27**: 173-180.

Malatesta *et al*. 2003. European Journal of Histochemistry, **47**(4): 385-88.

National Resources Defense Council (NRDC), USA. 1998. Health hazards of pesticides. A report published in October, 1998.

Nordlee *et al*. 1996. The New England Journal of Medicine **334**: 688-92

Pusztai, A. and Ewen S.W.B. 1999. Health risks of genetically modified foods. *Lancet*, *354*,684; and Ewen, S.W.B. and Pusztai, A.1999. Effects of diets containing genetically modified potatoes expressing *Galanthus nivalis lectin* on rat small intestine, *Lancet*, **354**,1353-4

Quist, D.A. and Chapela, I., 2001 Nature **414**, 541-543; (also see Dalton, R. 2008 Nature, **456**, 149).

Sarkar and others 2008. Transgenenic Bt Cotton affects enzymes and nutrients in Subtropical Inceptisol. Journal of Agronomy and Crop Science [ISSN 0931 – 2250].

Seralini, G.E., Mesnage, R., Clair, E., Gress, S., de Vendomois, J.S. and Cellier, D.2011. Genetically modified crops safety assessments: Present limits and possible improvements. Environmental Sciences Europe, 23: 10-20. Also available at http://www.enveurope.com/content/23/1/10.

Singh G.P.I 2009. Health issue related to genetically modified food and non-food crops, email: gpisingh@gmail.com

Smith Jeffrey, 2011. Monsanto's Roundup Triggers Over 40 Plant Diseases and Endangers Human and Animal Health, link: http://www.naturalnews.com/031138_Monsanto_Roundup.html#

Spiroux de Vendomois, J. *et al.* 2009. A comparison of the effects of three GM corn varieties on mammalian health. International Journal of Biological Sciences **5**(7): 706-26..

Statement by the Executive committee of the American Academy of Environmental Medicine on May 8,2009, available on http://www.aaemonl.com.

Velimirov, A., Binter,C and Zentek,J. 2008. Biological effects of transgenic maize NK 603 X MON 810 fed in long term reproduction in mice. November 2008 ISBN 978-3-902611-24-6.

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GERMINATION OF RICE SEEDS PRIMED WITH VARIOUS SALTS

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ABSTRACT

This study showed the effect of priming on seeds of two rice varieties (HUR3022 and Sahbhagi dhan) with salts $NaHCO_3$, KH_2PO_4, $Mg(NO_3)_2$ and K_2SO_4 used at various concentrations (range 5-20mM) on germination percent, dry weight and vigor index of seeds and seedlings respectively in relation to non primed control sets. Among the treatments the 5 to 10mM concentrations were found to improve seed germination parameters. $Mg(NO_3)_2$ priming showed maximum vigor index that has been followed by K_2SO_4, $NaHCO_3$ and KH_2PO_4. This study also showed a co-related relationship between increased á-amylase activity and levels of soluble sugar in the endosperm of primed seeds; the insoluble sugar content showed a reverse trend in respect to soluble sugar.

Key words: Priming, germination percentage, vigor index, á- amylase.

INTRODUCTION

Rice is a staple food for nearly half of the world's population. Rice is important for food security in several of the poorest countries; investments in the rice sector should be considered to improve poverty and meet the food demands of still rising and gradually more urbanized populations (Maclean *et al.*[16]). More than 90% of the world's rice is grown and consumed in Asia, The solution achieving food security and alleviating scarcity in rising rice productivity lies for lowering production costs. In this respect seed priming/hardening is widely used to enhance rate and uniformity of seed germination (Sharma and Bose[18], De Lespinay *et al.*[11]).

During unfavorable environmental condition it is difficult to achieve good quality crop establishment and maximum possible plant populations in the field by high-quality germination and emergence. Primed seeds generally shows an improved germination rate, greater germination uniformity, and, at times, greater total germination percent (Basra *et al.*[3]), increased germination under sub-optimal conditions (Lin and Sung[15]). Increased germination rate and uniformity have been ascribed to metabolic repair for the duration of imbibitions (Bray *et al.*[8]), increase of germination-enhancing metabolites (Basra *et al.*[3], Anayatullah and Bose[1]), osmotic adjustment (Bradford[7]), and for seeds that are not redried after treatment, a simple reduction in imbibition lag time (Bradford[7]). In recent times Bose and Mishra[5], Sharma and Bose[19] and Bose *et al.*[6] did a few work with nitrate seed priming/hardening technology where the seeds of different field crops such as wheat, maize and mustard were hardened with different nitrate salts [$(Mg(NO_3)_2$ and KNO_3] before sowing and observed an enhanced rate of germination physiology and vegetative growth. In seed priming technology seeds are treated with precisely controlled conditions to allow most events of germination to occur while preventing the emergence of radical from seed coat. To improve the quality of seeds by seed priming beside water a number of inorganic and organic chemicals including PGR are in use. Those are mainly KNO_3, KH_2PO_4, NaCl, $MgSO_4$, $NaHCO_3$, K_3PO_4 and $CaCl_2$ among these the anion part has found to have their response on the metabolism of plants like NO_3^- anion regulates the nitrogen metabolism, phosphate may be involve in energy metabolism, carbonate (CO_3^-) may do interfere with carbon metabolism, as well as sulphate has immense role in sulpher metabolism by which it can generate some important amino acids (Anayatullah and Bose[1]).With these views in mind in present investigation four easily available chemicals like K_2SO_4, $NaHCO_3$, KH_2PO_4 and $Mg(NO_3)_2$ were used for priming purposes to find out their influence on the germination physiology of rice.

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MATERIASL AND METHODS

Seeds of two rice varieties procured from the Institute of Agricultural Sciences were surface sterilized with 0.01% $HgCl_2$ solution (2 min.) then washed thoroughly with distilled water for 5-6 times. For priming treatment the seeds were either kept in beaker having varying concentrations [5,7.5,10,15 and 20 mM (C_2, C_3, C_4,C_5 and C_6)] of $NaHCO_3(S_1)$, $KH_2PO_4(S_2)$, $Mg(NO_3)_2(S_3)$ and K_2SO_4 (S_4) for 20 h. After that seeds were washed once with distilled water and dried back to the original weight by following the method described by Anayatullah and Bose[1] and now they were referred as primed seeds. The primed and non primed (NPC) control seeds then placed in petridishes (200 mm diameter) moistened with distilled water at a temperature of 30 ± 2°C in normal light condition. The observations were done as per requirement at different hours/days. The rate of germination and vigor index were measured by using following formula:

$$\text{Germination \%} = \frac{\text{No. of seeds germination x 100}}{\text{No. of seeds present in petridish}}$$

Vigor index = germination % x Dry wt. of 7 days old seedling

á-Amylase, soluble and insoluble sugar content of endosperm of the seeds, taken from the concentration of the different salts, showed best % of germination and vigor index were measured by using the method of Bernfeld[4] , Dubois *et al.*[12] respectively. The statistical analysis CRD factorial was implemented as per the experiment required.

RESULTS AND DISCUSSION

Table 1 and 2 represented that the salt $NaHCO_3$ treated sets showed maximum germination % and vigor index at 18 h for C_5 and C_6 treatments for HUR3022 (V_1) while in sahbhagidhan (V_2) variety control represented higher % germination at early hours (18, 24, 30 h) but with increasing time duration salt treated sets showed better performance in respect to it, whereas vigor index was always higher in primed sets than control in V_1 and V_2 both. For KH_2PO_4 at 18 h maximum germination percent (48%) and vigor index (1.27) was found for V_1 in C_4 and for V_2 control showed best but as germination hours increased vigor index also increased for both the varieties [C_5 (2.49) and C_6(1.99) at 48h]. In case of $Mg(NO_3)_2$ at 18h C_2 showed maximum germination percent (48%) for V_1 but in case of V_2 control was best but as time increased vigor index increased. For both the varieties at 48 h C_3 showed best result [2.65(V_1) and 2.07(V_2)]. K_2SO_4 treated sets noted to have best results for C_3 (96%) and C_4 (90.67%) concentrations at 48h for V_1 and V_2 respected and the maximum vigor was found in C_4 for both the varieties [2.46(V_1) and 2.58 (V_2)] (Table 1& 2). However the present study depicted that both varieties of rice HUR3022 and Sahbhagidhan showed maximum germination percentage and vigor index in $Mg(NO_3)_2$ (7.5mM) treated sets but a difference was observed in their time duration for maximum germination percent. The study of á- amylase activity of endosperm depicted that at 3 days of germination (DOG) it was more as compared to 4 DOG. Maximum activity of amylase enzyme at 3 DOG endosperm was represented by Mg $(NO_3)_2$ (0.305 mg maltose g^{-1} h^{-1} for V_1 and 0.292 mg maltose g^{-1} h^{-1} for V_2) salt followed by K_2SO_4 (0.267 mg maltose g^{-1} h^{-1} for V_1 and 0.254 mg maltose g^{-1} $h^{-1}$for V_2) while in 4 DOG endosperm the maximum activity was also observed for both the varieties in salt $Mg(NO_3)_2$ [0.122 mg maltose g^{-1} h^{-1}(V_1) and 0.119 mg maltose g^{-1} h^{-1}(V_2)] (Fig. 1(a). Whereas the soluble sugar content was noted to increase with increasing time and a concomitant reduction was observed in the contents of total insoluble sugar (Fig. 1(b) and (c). Soluble sugar content showed an upgrading in $Mg(NO_3)_2$ invigorated seeds with increasing hours of germination followed by K_2SO_4 treated one; NPC set was always found poor performer in this respect (0.136(V_1), 0.125(V_2) and 0.160 (V_1), 0.154 (V_2) mg g^{-1} in endosperm of 3 and 4 DOG) respectively (Fig. 1(b). The insoluble sugar content (Fig. 1(c) was found highest in case of $Mg(NO_3)_2$ (0.326(V_1), 0.455(V_2) and 0.293(V_1), 0.425(V_2) mg g^{-1}) in respect to other treatments at all the study periods. The study suggested that the best performance in this regard was observed with the $Mg(NO_3)_2$ primed sets followed by K_2SO_4, KH_2PO_4 and $NaHCO_3$ for both the varieties of rice. The non primed set was found to be poor performer for all the parameters studied.

It is clear from the present study that primed seeds in comparison with non primed seeds resulted in more seed germination percent and vigor index. The use of salt for priming can lead to greater vigor (Cantliffe[10]; Brocklehurts *et al.*[9]; Harris *et al.*[13]; Basra *et al.*[2]). Mondal *et.al.*[17] observed that $Mg(NO_3)_2$ hardened seeds of rice variety swarna (MTU7029) has better germination percentage and activity of á-amylase enzyme in endosperm over hydroprimed and nonprimed sets. Basra *et al.*[3] was reported that osmopriming increase germination and vigor in rice. Ruan *et al.*[18] used $CaCl_2$, KNO_3 and NaCl for priming and found that all the salts used for osmoconditioning significantly improved the

Table 1.

Effect of seed priming with different salts on germination percentage of two rice var. HUR3022(V_1) and Sahbhagidhan (V_2) at different study periods of germination. S_1: $NaHCO_3$, S_2:KH_2PO_4, S_3: $Mg(NO_3)_2$, S_4: K_2SO_4, NPC: non primed control, C_2:5mM, C_3:7.5mM, C_4: 10 mM, C_5:15mM, C_6:20mM.

SALT	CONC.	GERMINATION %													
		18 HR		24HR		30HR		36HR		42HR		48HR		V1	V2
		V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	mean	mean
S_1	NPC	12.0	49.3	66.6	65.3	81.3	76.0	93.3	85.3	93.3	88.0	93.33	88.00	73.31	75.32
	C_2	33.3	13.3	61.3	37.3	78.6	46.6	86.6	60.0	85.3	64.0	86.67	76.00	71.96	49.53
	C_3	48.0	18.6	61.3	36.0	74.6	44.0	81.3	60.0	88.0	65.3	88.00	81.33	73.53	50.87
	C_4	53.3	12.0	74.6	41.3	85.3	50.6	88.0	65.3	88.0	78.6	88.00	82.67	79.53	55.08
	C_5	56.0	16.0	73.3	38.6	84.0	48.0	89.3	62.6	89.3	76.0	90.67	80.00	80.43	53.53
	C_6	56.0	13.0	81.3	45.3	84	56.0	96.0	70.6	96.0	76.0	96.00	85.33	84.88	57.71
S_2	NPC	12.0	49.3	66.6	65.3	81.3	76.0	93.3	85.3	93.3	88.0	93.33	88.00	73.31	75.32
	C_2	24.0	13.3	64.0	37.3	78.6	45.3	58.6	68.0	68.0	69.3	77.33	77.33	61.76	51.76
	C_3	29.0	20.0	60.0	49.3	76.0	54.6	64.0	64.0	77.3	76.0	80.00	81.33	64.38	57.54
	C_4	48.0	21.3	70.6	45.3	76.0	53.3	80.0	78.6	76.0	74.6	80.00	80.00	71.77	58.85
	C_5	36.0	10.6	45.3	52.0	61.3	50.6	76.0	73.3	78.6	77.3	82.67	80.00	63.31	57.30
	C_6	36.0	20.0	38.6	58.6	64.0	57.3	76.0	74.0	84.0	80.0	89.33	85.33	64.66	62.54
S3	NPC	12.0	49.3	66.6	65.3	81.3	76.0	93.3	85.3	93.3	88.0	93.33	88.00	73.31	75.32
	C2	48.0	13.3	73.3	38.6	82.6	45.3	93.3	58.0	93.3	66.6	93.33	77.33	80.64	49.86
	C3	44.0	20.0	80.0	49.3	89.3	54.6	93.3	64.0	93.3	76.0	93.33	81.33	82.21	57.54
	C4	40.0	21.3	80.0	46.6	86.6	56.0	89.3	78.6	89.3	77.3	89.33	80.00	79.09	59.97
	C5	33.3	10.6	73.3	38.6	81.3	53.3	88.0	66.6	88.0	77.3	88.00	80.00	75.32	54.40
	C6	45.3	20.0	74.6	42.6	85.3	50.6	92.0	77.3	92.0	82.6	90.67	85.33	79.98	59.74
S4	NPC	12.0	49.3	66.6	65.3	81.3	76.0	93.3	85.3	93.3	88.0	93.33	88.00	73.31	75.32
	C2	9.3	21.3	32.0	13.3	53.3	36.0	82.6	88.0	88.0	89.0	88.00	89.33	58.87	56.16
	C3	14.6	28.0	32.0	29.3	64.0	48.0	88.0	89.0	88.0	90.6	96.00	89.33	63.77	62.37
	C4	10.6	14.6	36.0	17.3	72.0	68.0	93.3	89.3	93.3	90.6	93.33	90.67	66.42	61.75
	C5	16.0	13.3	29.3	17.3	65.3	40.0	84.0	88.0	88.0	88.0	88.00	88.00	61.77	55.77
	C6	10.6	16.0	28.0	16.0	65.3	49.0	80.0	76.0	89.3	89.3	89.33	89.33	60.42	55.94

Particulars (C.D. of the factors at 5%)	18 HR	24HR	30HR	36HR	42HR	48HR
A	1.7157	2.0729	2.3257	2.8409	2.3038	2.2876
B	2.1013	2.5387	2.8484	3.4794	2.8216	2.8017
C	1.2132	1.4657	1.6445	2.0089	1.6290	1.6176
AxB	4.2026	5.0775	5.6967	6.9589	5.6431	5.6034
AxC	2.4264	2.9315	3.2890	4.0177	3.2581	3.2351
BxC	2.9717	3.5903	4.0282	4.9207	3.9903	3.9622
AxBxC	5.9434	7.1806	8.0564	9.8413	7.9806	7.9244

Table 2.

Effect of seed priming with different salts on vigor index of two rice var. HUR3022(V_1) and Sahbhagidhan (V_2) at different study periods of germination. S_1: $NaHCO_3$, S_2:KH_2PO_4, S_3: $Mg(NO_3)_2$, S_4: K_2SO_4, NPC: non primed control, C_2:5mM, C_3:7.5mM, C_4: 10 mM, C_5:15 mM, C_6:20mM.

SALT	CONC.	GERMINATION %													
		18 HR		24HR		30HR		36HR		42HR		48HR		V1	V2
		V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	mean	mean
S_1	NPC	0.34	1.09	1.53	1.47	1.83	1.67	1.83	1.87	1.88	1.93	1.86	1.93	1.55	1.66
	C_2	0.74	0.23	1.36	5.99	1.74	0.80	1.92	1.08	1.89	1.21	1.92	1.34	1.60	1.78
	C_3	0.89	0.46	1.14	1.13	1.38	1.21	1.51	1.53	1.63	1.74	1.65	1.85	1.37	1.32
	C_4	1.21	0.46	1.69	6.04	1.93	1.20	1.99	1.56	1.98	1.74	1.99	1.87	1.80	2.15
	C_5	0.81	0.24	1.06	8.82	1.21	1.09	1.29	1.48	1.29	1.77	1.31	1.82	1.16	2.54
	C_6	1.22	0.37	1.33	6.75	1.42	1.19	1.52	1.45	1.61	1.86	1.63	1.99	1.46	2.27
S_2	NPC	0.34	1.09	1.53	1.47	1.83	1.67	1.84	1.87	1.87	1.93	1.86	1.93	1.55	1.66
	C_2	0.55	0.23	1.48	0.59	1.81	0.79	1.35	1.08	1.57	1.21	1.78	1.34	1.42	0.87
	C_3	0.86	0.42	1.75	1.12	2.23	1.25	1.87	1.46	2.26	1.74	2.34	1.85	1.89	1.31
	C_4	1.27	0.49	1.87	0.97	2.01	1.24	2.12	1.62	2.01	1.74	2.11	1.87	1.90	1.32
	C_5	1.09	0.24	1.57	0.88	1.85	1.16	2.29	1.61	2.37	1.77	2.49	1.82	1.94	1.25
	C_6	0.98	0.46	1.60	1.05	1.74	1.33	2.07	1.71	2.29	1.86	2.44	1.99	1.85	1.40
S_3	NPC	0.34	1.09	1.52	1.47	1.83	1.67	1.83	1.87	1.87	1.93	1.86	1.93	1.54	1.66
	C_2	1.09	0.25	1.73	1.04	1.98	1.04	2.26	1.75	1.98	1.88	2.49	1.90	1.92	1.31
	C_3	1.25	0.25	2.27	1.36	2.54	1.48	2.65	1.75	2.54	2.04	2.65	2.07	2.32	1.49
	C_4	1.08	0.39	2.00	1.11	2.13	1.48	2.20	1.67	2.13	1.87	2.56	2.07	2.02	1.43
	C_5	0.85	0.21	1.88	1.02	2.08	1.30	2.25	2.32	2.08	1.98	2.25	1.88	1.90	1.45
	C_6	0.63	0.29	1.54	0.83	1.75	1.18	1.89	1.57	1.75	1.79	1.86	1.82	1.57	1.25
S_4	NPC	0.34	1.09	1.52	1.47	1.83	1.68	1.83	1.87	1.87	1.93	1.86	1.93	1.54	1.66
	C_2	0.20	0.46	0.71	0.96	1.19	2.20	1.85	2.28	1.97	2.38	1.97	2.38	1.32	1.78
	C_3	0.30	0.64	0.65	1.15	1.31	1.93	1.80	2.20	1.80	2.30	1.96	2.30	1.30	1.75
	C_4	0.28	0.46	0.94	1.83	1.90	2.51	2.46	2.54	2.46	2.58	2.46	2.58	1.75	2.08
	C_5	0.37	0.47	0.68	1.17	1.52	2.19	1.95	2.58	2.04	2.58	2.04	2.58	1.43	1.93
	C_6	0.25	0.46	0.65	1.44	1.53	2.28	1.87	2.39	2.09	2.55	2.09	2.55	1.41	1.95

Particulars (C.D. of the factors at 5%)	18 HR	24HR	30HR	36HR	42HR	48HR
A	0.1088	.4491	0.0520	0.0635	0.0457	0.0486
B	0.1332	.5500	0.0637	0.0777	0.0560	0.0595
C	0.0769	.3175	0.0368	0.0449	0.0323	0.0344
AxB	0.2667	1.1000	0.1275	0.1555	0.1119	0.1190
AxC	0.1538	0.6351	0.0736	0.0898	0.0646	0.0687
BxC	0.1884	0.7778	0.0902	0.1099	0.0791	0.0841
AxBxC	0.3768	1.5556	0.1803	0.2199	0.1583	0.1683

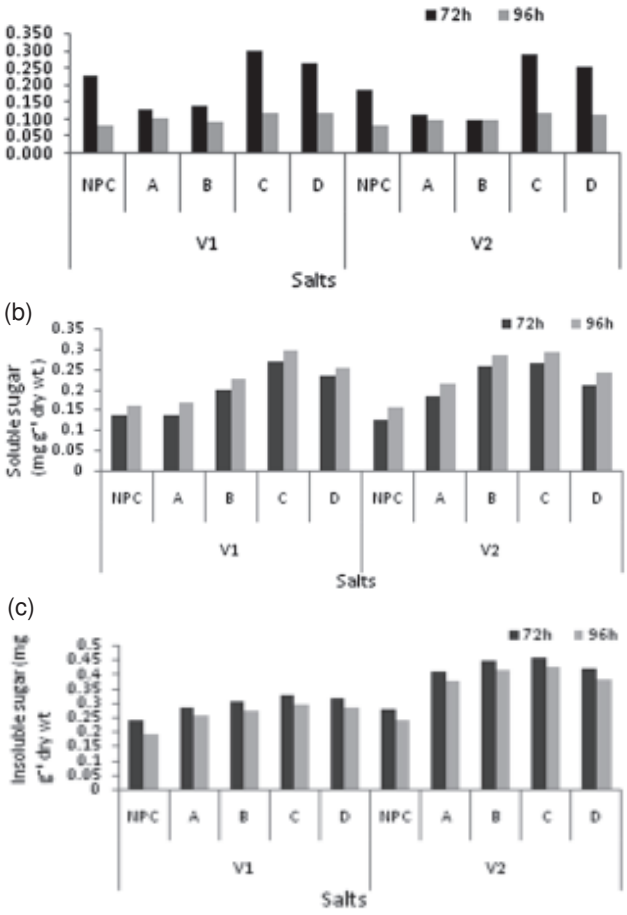

Fig. 1. Effect of seed priming with different salts, (most efficient concentration) on á-Amylase Activity (**a**), soluble (**b**) and insoluble sugar (**c**) content of two rice var. HUR3022(V_1) and Sahbhagidhan (V_2) at different study periods of germination. NPC: non primed control, A: $NaHCO_3$, B: KH_2PO_4, C: $Mg(NO_3)_2$, D: K_2SO_4.

rate and speed of germination and seedling vigor in rice. Lee and Kim[14] also reported an increase in á-amylase and total sugar content in primed seed ; the same was found in the present study (Fig.1 (a)and (b). The present study concludes that although for priming a number of salts are in use for different crops but it seems that the $Mg(NO_3)_2$ may be a better option for priming purposes of rice varieties specifically for HUR3022 and Sahbhagi dhan in respect to other used salts. This may be due to the action of Mg^{+2} and NO_3^- ions present in this salt; regarding this the studies are in continuation.

REFRENCES

1. Anaytullah and B. Bose, *Physiol. Mol. Biol. Plants,* **13**:199-207 (2007).
2. Basra, S.M.A., Ehsanullah, E., Warraich, A., Cheema, M.A. and I. Afzal, *Int. J. Agric. Bio*., 117-120 (2003).
3. Basra, S.M.A., Farooq, M. and R. Tabassum, *Seed Sci. Technol.* **33**: 25-29(2005).
4. Bernfeld, P. *Methods in Enzymology*, **1**: 149-158 (1955).
5. Bose, B. and T. Mishra, *In. J. Plant Physiol.,* **6**: 431-434 (2001).
6. Bose, B., Kumar, R., Kuril, S.K. and A.K. Shrivastava, *Brassica*, **9**: 33-38(2007).
7. Bradford, K.J., *Hort. Sci., Alexandria*, **21**: 1105-1112 (1986).
8. Bray, C.M., Davison, P.A., Ashraf, M. and R.M. Taylor, *Ann. Bot.,* **63**: 185-193(1989).
9. Brocklehurst, P.A., Dearman, J. and R.L.K.Drew, *Acta Hort.*, **215**: 193-200 (1987).
10. Cantliffe, D.G., *J. Korean Soc. Hort. Sci.,* **38**: 441- 445 (1997).
11. De Lespinay, A., Lequeux, H., Lambillotte, B. and S. Lutts, *Plant Growth Regul*., **61**: 205-214 (2010).
12. Dubios, M., Gilles, R.A., Hamilton, J.K., Robevls, P.A. and F. Smith, *Anal. Chem*., **28**: 350-356 (1956).
13. Harris, D., Pathan, A.K., Gothkar, P., Joshi, A., Chivasa, W. and P. Nyamudeza, *Agric. Syst*., **69**: 151-164 (2001).
14. Lee, S.S. and J.H. Kim, *Korean J. Crop Sci*., **45**: 108-111(2000).
15. Lin, J.M. and Sung, J.M. *Seed Sci. Technol.,* **29**: 39-50 (2001).
16. Maclean, J.L., Dawe, D. and B. Hardy, *Rice Almanac.* Hettel, G.P. eds. 3rd ed. Wallingford, UK: CABI Publishing. (2002).
17. Mondal, S., Vijai, P. and B. Bose, *Research Journal of Seed Science,* **4**: 157-165 (2011).
18. Ruan, S., Xue Q. and K. Tylkowska. *Seed Sci. Technol.*, **30**: 61-67 (2002).
19. Sharma, M.K. and B. Bose, *Physiol. Mol. Biol. Plants*. **12**: 173-176 (2006).

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TECHNIQUE FOR INVIGOURATION OF SEEDS OF A LENTIL CULTIVAR (*Lens esculenta* L. CV. WBL-77) AND ITS EXPEDITIOUS EVALUATION BY ACCELERATED AGEING

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ABSTRACT

An experiment was designed for establishment of a farmer-friendly simple herbal manipulative method for invigouration of crop seeds under storage. Treatment-induced invigouration of lentil (*Lens esculenta* L. cv. WBL-77) seeds was expeditiously evaluated by employing accelerated ageing technique. Seed pretreatment with aqueous leaf extracts of *Ocimum sanctum* and *Aegle marmelos* enhanced seed germination behaviour over control sample with concomitant reduction of deleterious leaching of electrolytes and soluble carbohydrates of the seeds which were stored under stressful accelerated ageing condition. Such changes were associated with the treatment-induced significant enhancement of the activities of dehydrogenase and catalase over control. The parameters recorded are indicative of higher potential status of seeds which experienced pretreatment with the herbal agents, justifying their effect on seed invigouration even under adverse storage.

INTRODUCTION

Most crop seeds require storage for either one or several planting seasons for better field performance. But seed storage may adversely affect vigour status of seeds and thus the viability span of the seeds is reduced depending upon the storage condition and duration. Adverse environmental conditions like high temperature and high relative humidity (RH), prevailing in India particularly in West Bengal during the major parts of a year, cause degradation of seed quality. These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability, field emergence capacity as well as field performance of seedlings and plants leading to impairment of productivity (Desai *et al.,* 1997; Maity *et al.,* 2000; Bhattacharjee, 2001; Copeland and McDonald, 2001; Bhattacharjee *et al.,* 2004; Das, 2008). Keeping in mind such problems, an attempt was made for storage hardening of seeds of a low vigour lentil cultivar (*Lens esculenta* L. cv. WBL-77) by herbal manipulation technique. In fact, this technique is bio-friendly and nonhazardous over chemical manipulation for enhanced seed storage potential.

Thus, the prime objective of this work was to assess the effects of selected herbal agents, after an initial screening, on seed invigouration of the experimental lentil cultivar on the basis of some reliable physiological and biochemical methods. In fact, invigourated seeds are considered as superior seed quality in terms of seed germinability, field performance and consequent productivity of crops.

MATERIALS AND METHODS

Lentil seeds (*Lens esculenta* L. cv. WBL-77) were procured from Bidhan Chandra Krishi viswavidyalaya, Mohanpur, Nadia and before herbal treatment these were surface sterilized using 0.1% mercuric chloride ($HgCl_2$) for 90 seconds. Seeds were then pre-treated with aqueous solutions of *Ocimum sanctum* and *Aegle marmelos* leaf extracts (25% and 50% each) and distilled water for 6 hours and then dried back to their original weight. This was repeated twice and finally seeds were brought back to the original moisture level. The pre-treated seed lots were then subjected to accelerated aging treatment (99.1% RH) for 0, 15 and 30 days, and this is adverse artificial stress storage environment created within a desiccator containing 3.03% sulfuric acid. In fact, such stressful storage environment expeditiously and accurately determine the vigour status of crop seeds (Heydecker, 1977).

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Seed germinability (%) was recorded along with the determination of T_{50} (time required for 50% seed germination, in hour') values of seed germination. From seed kernels leaching of electrolytes (Choudhury and Basu, 1988), soluble carbohydrate (McCready *et al.*, 1950), dehydrogenase (Rudrapal and Basu, 1979) and catalase (Snell and Snell, 1971, modified by Biswas and Choudhuri, 1978) activities were recorded. Data recorded in this investigation at the treatment and replication levels and statistical analysis of the data, presented in the tables, was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits as per the method of Panse and Sukhatme (1967).In figures each bar represents the average results of three replicates.

RESULTS AND DISCUSSION

Seed pre-treatment with the two herbal agents (25% and 50% each) positively influenced germination behavior recorded in terms of higher percentage of germination and lesser time required for attainment of 50% germination when the seeds experienced with the herbal treatments prior to accelerated ageing (Table 1) and best response was recorded at 25% leaf extract of *Ocimum sanctum* in respect of enhanced germinability.

Rapid leaching of electrolytes from seeds was recorded in all the seed samples which underwent accelerated ageing for longer duration. The herbal treatments, regardless of their concentrations, significantly ameliorated the deleterious electrolyte leaching (Table 2) and the magnitude of amelioration was found to be higher at later ageing days. Like electrolyte leaching the result of soluble carbohydrate (Table3) was found to be more or less identical and herbal agents were found potential enough for reducing the level of soluble carbohydrates in the plant extract treated seed samples. The treatment-induced higher activities of both dehydrogenase and catalase (Table 4) were encountered. Here also, most significant increase of the enzyme activities in the plant extract-treated seed samples against control values were recorded after 30 days of ageing experiment.

There are numerous reports that decrease in membrane integrity as well as enzyme activities play a significant role in the deterioration of seeds leading to loss of viability (Abdul Baki and Anderson, 1972; Powell and Matthews, 1977; Francis and Coolbear, 1984; Bhattacharjee and Choudhuri, 1986; Mishra *et al.*, 2003). In fact, biochemical parameters are increasingly used as

Table 1.

Effect of seed pretreatment with leaf extracts of *Ocimum sanctum* and *Aegle marmelos* on percentage (%) germination and T_{50} (time required for 50% germination in hours) of lentil seeds under stressful storage condition (99.1% RH)

Treatments		Seed storage period (day)	% germination	T_{50} values
Control		0	90.0	24
		15	60.9	140
		30	40.5	NA
Ocimum	25%	0	95.5	24
		15	75.9	92
		30	50.3	168
	50%	0	85.5	24
		15	65.5	116
		30	45.5	NA
Aegle	25%	0	90.8	24
		15	70.0	92
		30	45.0	212
	50%	0	85.5	24
		15	60.5	116
		30	40.5	NA
LSD (P=0.05)			3.87	6.79

NA: Nonattainment of 50% germination.

Table 2.

Effect of seed pretreatment with leaf extracts of *Ocimum sanctum and Aegle marmelos* with two different concentrations on leaching of electrolytes (μ MHO) in lentil seeds under stressful storage condition (99.1% RH)

Treatments		Treatment period (day) under R.H	Leaching of electrolytes		
			1 hour	3 hours	24 hours
Control		0	15.18	18.08	30.81
		15	19.58	24.82	42.83
		30	25.15	30.98	48.84
Ocimum	25%	0	13.28	18.41	30.21
		15	14.37	19.82	33.86
		30	17.67	29.88	47.81
	50%	0	11.14	17.08	25.41
		15	13.92	19.86	40.31
		30	18.67	31.98	43.81
Aegle	25%	0	17.29	21.98	28.51
		15	19.50	24.79	34.21
		30	24.41	30.88	41.71
	50%	0	15.64	21.51	26.71
		15	17.40	22.84	36.41
		30	20.10	37.62	44.51
LSD (P=0.05)			1.02	1.36	2.70

Table 3.

Effect of seed pretreatment with leaf extracts of *Ocimum sanctum and Aegle marmelos* with two different concentrations on leaching of total soluble Carbohydrates (μg/g/100ml) in lentil seeds under stressful storage condition (99.1% RH)

Treatments		Treatment period (day) under R.H	Soluble carbohydrate		
			1 hour	3 hours	24 hours
Control		0	21.48	30.26	50.37
		15	27.37	41.17	57.23
		30	33.45	47.35	61.18
Ocimum	25%	0	13.76	22.33	43.02
		15	19.88	29.55	50.47
		30	23.30	34.15	53.68
	50%	0	17.57	26.76	47.00
		15	22.30	35.16	53.31
		30	26.16	38.25	57.20
Aegle	25%	0	15.57	25.76	44.77
		15	20.57	32.15	51.98
		30	24.88	36.41	55.64
	50%	0	21.47	29.63	48.20
		15	26.12	38.59	55.31
		30	30.1	43.51	59.70
LSD (P=0.05)			1.45	2.03	4.24

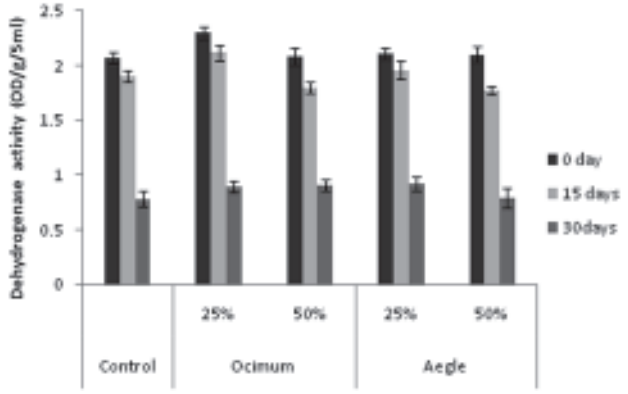

Fig. 1. Effect of seed pretreatment with leaf extracts of *Ocimum sanctum* and *Aegle marmelos* on changes in dehydrogenase (OD/g/5ml) activity of lentil seeds under stressful storage condition (99.1% RH)

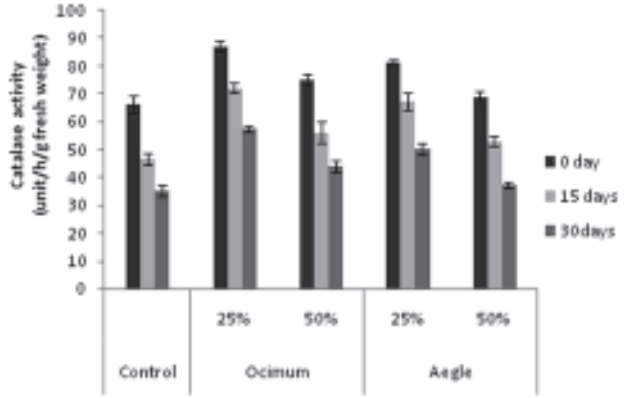

Fig. 2. Effect of seed pretreatment with leaf extracts of *Ocimum sanctum* and *Aegle marmelos* on changes in catalase activity (unit/h/g fresh weight) of lentil seeds under stressful storage condition (99.1% RH).

indicators of seed viability and vigour status (Ramesh and Ramprasad, 2013; Pulok *et al.*, 2015).Our results on the plant extract induced changes of some biochemical parameters corroborate the observations of previous reports. Thus, it can be concluded that the test seed pretreating agents (*Ocimum sanctum* and *Aegle marmelos* leaf extracts) are effective for storage hardening and invigouration of the seeds of the experimental lentil cultivar. The herbal treatments can significantly harden seeds even under adverse storage condition, thereby causing invigouration of the lentil seeds and thus making them less susceptible towards deterioration under adverse ambient storage situation prevailing in India. An improvement of productivity is likely to possible using such invigourated seeds and research is in progress on this aspect in field-based experiment.

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REFERENCES

Abdul-Baki, A. A. and Anderson, J. D. (1972). Physiological and biochemical deterioration of seeds. In *Seed Biology*, (ed. T. T. Kozlowski), vol. 2. pp. 203-215, Academic press, New York.

Biswas, A. K.and Choudhuri, M. A. (1978). Differential behaviour of the flag leaf of intact rice plant during ageing. *Biochemie und Physiologie der Pflanzen*, **173**, 220-228.

Bhattacharjee, A. and Choudhuri, M. A. (1986). Chemical manipulation of seed longevity and stress tolerance capacity of seedlings of *Corchorus capsularis* and *C. olitorius*. *Journal of Plant Physiology*, **125**, 391- 400.

Choudhuri, N. and Basu, R. N. (1988). Maintenance of seed vigour and viability of onion (*Allium cepa* L.). *Seed Science and Technology*, **16**, 51-61.

Das, R. K. (2008). Ph.D. Thesis. Investigation on the influence of plant extracts of neem (*Azadirachta indica*), kalmegh (*Andrographis paniculata*) and asoka (*Saraca asoka*) on storage potentiation of seeds.

Francis, A. and Coolbear, P. (1984). Changes in the membrane phospholipid composition of tomato seeds accompanying loss of germination capacity caused by controlled deterioration. *Journal of Experimental Botany*, **35**, 161-170.

Heydecker, W. (1977). Stress and seed germination: an agronomic view. In *Physiology and Biochemistry of Seed Dormacy and Germination*. (ed. A.A. Khan). Elsevier, North Holland, Amsterdam, pp. 237-282.

McCready, R. M., Guggloz, J., Silviera, V. and Owens, J. S. (1950). Deterioration of starch and amylase in vegetables. *Analytical Chemistry*, 22, 1156-1158.

Mishra, R. K. and Sahoo N. C. (2003). Effect of chemical priming and ageing on seed vigour, viability and enzyme activity in tomato and cauliflower. *Indian Journal of Plant Physiology*, Spl. Issue pp. 222-225.

Moore, R. P. (1973). Tetrazolium staining for assessing seed quality. In *Seed Ecology* (ed. W. Heydeker). p. 347. Butterworths, London.

Panse, V. G. and Sukhatme, P. T. (1967). *Statistical Methods for Agricultural Workers*.2nd edition, pp. 150-157. Indian Council of Agricultural Research, New Delhi, India.

Powell, A. A. and Mathews, S. (1977). Deteriorative changes in pea seeds in humid or dry conditions. *Journal of Experimental Botany*, **28**, 225-234.

Pulok, M. A. I., Rahman, M. M., Haque, M. N., Chakraborty, R. and Ali, M. S. (2015). Effect of growth regulators on germination and vigor of lentil seeds. *Journal of Bioscience and Agriculture Research*. Vol. **03**(01): 08-14.

Ramesh, R.and Ramprasad, E. (2013). Effect of plant growth regulators on morphological, physiological and biochemical parameters of soybean (*Glycine max* L. Merrill). *Helix.* Vol. **6**: 441 – 447.

Rudrapal, A. B. and Basu, R. N. (1979). Physiology of hydration-dehydration treatments in the maintenance of seed viability in wheat. *Indian Journal of Experimental Biology.* **17**, 768-771.

Snell, F. D. and Snell, C. T. (1971). *Colorimetric Methods of Analysis*, Vol. 4AAA. p 7-145, Van Nostrand Reinhold Co., New York.

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ALLELOPATHIC EFFECT OF KALMEGH SEED EXTRACT OVER VARIOUS CEREAL AND PULSE SEED GERMINATION BECOMES BENEFICIAL IF DILUTED

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ABSTRACT

Kalmegh (*Andrographis paniculata*) is a well proved medicinal herb in homoeopathic and ayurvedic medicinal system. There is high demand for this medicinal herb in market but it faces seed germination problem may be due to presence of some germination inhibitors in seed. Observing its medicinal efficacy against various human diseases attempts have been made to use it as plant disease curing medicine by several researchers. Taking the above in view we tried to find it out whether any germination inhibiting ability is there in crude seed extract of kalmegh over other crop seeds or not and then the extract gradually made diluted following the decicimal method as described by HPI (Homoeopathic Pharmacopia of India) upto 10^{th} level of dilution to observe its effect over seed germination and seedling behavior of the same crop seeds (Rice, Wheat, Gram, Lentil and Moong) in laboratory conditions. Result shows that germinations were completely blocked in case of pulses where as the rate and percentage were decreased in cereals. Dilutions affected the germination and seedling properties positively, increased seedling vigor and viability over control.

INRODUCTION

Kalmegh (*Andrographis paniculata)* is a potential medicinal herb used in homoeopathy and ayurvedic medicinal system for a long time. It is used as anti-inflammatory, antibacterial, antiperiodic, antipyretic, antithrombotic, antiviral, hypoglycemic, hepato protective, choleretic medicine (Kapil *et al.* 1993). Even its property as anticarcinogenic is well established, specially against colon cancer (Puri & Suxena 1993). In modern investigation the andrographolide (the main active ingredient) shows antineoplastic effect due to its ability to arrest cell cycle and control apoptosis (Varma *et. al.* 2009). Kalmegh has high market demand but faces seed germination problem (Kumar, 2011) that hampers agricultural practice. Several attempts have been made to overcome the seed dormancy. Medicinal Plants are not only useful to cure our diseases rather they also have some active ingredients which provide protective measures to plant diseases also. Some active principles like azadirectin of *Azadirecta indica*, Nicotin of *Nicotiana tabacum*, rotenon of *Derris* sp, Pyrithrin of *Chrysanthmum* sp are well known biopesticides which are use to control different pests in sustainable agriculture. They may be cultivated with other crops by intercropping management or for crop rotation (Nair G.S. 1991). As for example Pegion Pea/Maize + *Andrographis* / *Chlorophytum* intercropping system were proved to be advantageous over soul cropping (Yaseen M, Tripathi R S, 2009). Pre sowing seed treatment by herbal extracts in lentil improved its vigour and productivity in heat stressed condition (D. Roy Chowdhury *et al.* 2013), in rice seeds improved vigor and productivity (S. Kundu *et al.* 2013).

Taking above in view our present research plan was developed in such a way that first, we can investigate presence of any germination inhibitor in Kalmegh seeds and then secondly, making the Kalmegh seed extract beneficial in case of germination and seedling behaviour of different economically important crops (rice, wheat, chickpea, moong and gram) by diluting the crude extract, because crude phytotoxic substances converted beneficial by decreasing their concentration (Ma *et al.* 2011) over other crops and it is a regular process in homoeopathic pharmacy (Homoeopathic Pharmacopia of India).

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MATERIALS AND METHODS

15 gm. Of Kalmegh seed was dissolved in methanol for 3 days, then methanol was evaporated to dryness, then 150 ml. of water was added to the seed in same container, left for 2 days and the seed extract was filtered to get the crude treatment (T_1).

Then the crude extract(T_1) was gradually diluted in decicimal method (following Homoeopathic Pharmacopia of India) upto 10th level of dilution; 9 separate dilutions were prepared by taking 1 part of previous level and 9 part of water in successive levels.

Seeds of cereals (Rice and Wheat) and pulses (Gram, Lentil and Moong) were placed for germination in petridish with different treatments ($T_1, T_2, \ldots\ldots, T_{10}$) and in water as control in laboratory conditions. Germination percentage was calculated for successive 6 days for rice and 4 days for wheat, moong, gram and lentil. After 10 days of emergence of radicle germinated seeds were placed in aquaculture (tap water) upon the thermocol in such a way that the roots of the seeds were inserted through the hole of the thermocol and roots were touched the water. After seven days the following parameters were recorded.

Seedling root length, Seedling shoot length, Seed vigor index (following the formula)

Seed vigor index = (seedling root length + seedling shoot length) x germination % (Abdul Baki and Anderson, 1973), seedling fresh weight, seedling dry weight.

RESULT AND DISCUSSIONS

Effect of Kalmegh seed extract over cereal and pulse seeds were observed repeatedly in laboratory conditions and averaged to get the results. Paddy seeds were not much affected by Kalmegh seed extract though the germination percentage felled down to 53.3% in crude extract in respect of control (93.3%) (Table 1), where as T5, T8 and T9 treatments improved the germination percentage upto 100% over control. Like Paddy, Wheat was not also affected much but obviously rate and percentage of germination were slowed down in crude extract and improved little bit in T2, T5 and T10 in respect of control (Table 2), the dilution showed enough variations in seed germination too. The pulse seeds were more sensitive to Kalmegh extract than cereals (Table 3, 4 and 5). In crude extract germination was completely blocked in case of Lentil and Gram where as was decreased upto 50% in Moong, but in dilutions germination percentage increased over control effectively.

After observing seed germination percentage the behavior of seedlings including root - shoot length, fresh and dry weight and seed vigor were noticed of the germinated seeds in hydroponics; variations were evident in different dilutions of the Kalmegh seed extract. Pulses were more affected by treatments than cereals. In Paddy (Table 6), root length was enhanced in respect of control but shoot length minimized; both fresh and dry weight were not much affected; seed vigor in most of the cases fallen down except T5. In Wheat (Table 7), root length in all treatments were highly decreased where as shoot lengths were enhanced effectively; fresh and dry weight were also increased in some treatment conditions, specially in T3, T5 and T7; the seed vigor decreased in all treatments but significantly increased in T10.

In Moong (Table 8), root lengths were decreased in most of the cases but increased in treatment T8 and T10; shoot lengths also increased upto a good variation in T2, T4, T3, T8 and T10 (rank wise) but very low in crude extract treatment condition; seed vigor were decreased in many treatments but enough enhanced in T8, T9 and T10; fresh and dry weight were not affected much in respect of control. In Lentil (Table 9), treatment effects were evident enough. Root and shoot lengths were highly

Table 1.

Effect of crude and diluted form of Kalmegh seed extract on germination % of Rice

Successive Days of observation	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Day 1	0	0	0	0	0	0	0	0	0	0	0
Day 2	40	0	53.3	40	13.3	33.5	26.6	13.3	26.67	33.5	26.6
Day 3	80	13.3	80	60	73.3	80	80	73.3	93.3	93.3	66.6
Day 4	93.3	33.5	93.3	66.6	80	100	93.3	93.3	100	100	86.6
Day 5	93.3	53.3	93.3	73.3	80	100	93.3	93.3	100	100	93.3
Day 6	93.3	53.3	93.3	73.3	80	100	93.3	93.3	100	100	93.3

Table 2.

Effect of crude and diluted form of Kalmegh seed extract on germination % of Wheat

Successive Days of observation	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Day 1	15	0	15	20	25	35	20	15	40	45	35
Day 2	35	20	50	55	45	50	30	35	40	45	50
Day 3	50	45	65	55	50	60	35	35	45	50	60
Day 4	50	45	65	55	50	60	35	35	45	50	60

Table 3.

Effect of crude and diluted form of Kalmegh seed extract on germination % of Moong

Successive Days of observation	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Day 1	100	40	70	70	90	100	100	100	90	100	100
Day 2	100	50	70	100	90	100	100	100	100	100	100
Day 3	100	50	70	100	100	100	100	100	100	100	100
Day 4	100	50	70	100	100	100	100	100	100	100	100

Table 4.

Effect of crude and diluted form of Kalmegh seed extract on germination % of Lentil

Successive Days of observation	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Day 1	50	0	50	70	70	50	20	60	40	50	70
Day 2	50	0	60	70	80	60	40	70	50	60	80
Day 3	50	0	60	70	80	60	40	70	50	60	80
Day 4	50	0	60	70	80	60	40	70	50	60	80

Table 5.

Effect of crude and diluted form of Kalmegh seed extract on germination % of Gram

Successive Days of observation	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Day 1	30	0	0	30	50	50	10	40	20	0	20
Day 2	80	0	20	40	70	70	40	70	30	20	70
Day 3	80	0	20	40	70	70	40	70	30	20	70
Day 4	80	0	20	40	70	70	40	70	30	20	70

increased in almost all dilutions, specially in T6 and T9; fresh and dry weight were significantly increased in dilutions over control and best in T6 and T9; seed vigor was increased in high values, it was almost double in T10 and T4 than control. In Gram (Table. 10), root length was increased in all dilution treatments that was

Table 6.

Effect of crude and diluted form of Kalmegh seed extract on seedling behavior of Rice

Parameters	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Root length (cm.)	8.8	7.8	8.5	9.25	11.4	9.8	9.8	11	4.8	8.4	5.8
Shoot length (cm.)	9.6	9	7.25	7.25	9	7.6	8.6	6.8	2.4	5.8	6.4
Seed vigor	1716.7	895.44	1469.4	1209.9	1632	1740	1716.7	1660.7	720	1420	1138.2
Fresh weight (gm.)	0.032	0.034	0.0175	0.035	0.0278	0.0336	0.096	0.022	0.028	0.0166	0.0272
Dry weight (gm.)	0.009	0.006	0.009	0.008	0.008	0.008	0.008	0.007	0.002	0.004	0.007

Table 7.

Effect of crude and diluted form of Kalmegh seed extract on seedling behavior of Wheat

Parameters	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Root length (cm.)	10.87	10.25	5.7	5.6	7.44	6.1	6.26	6.675	6.3	4.675	8.7
Shoot length (cm.)	10.37	10.25	10	12.1	13.4	10.64	10.5	10.45	12.04	9.075	11.34
Seed vigor	1062	922.5	1020.5	973.5	1042	1004.4	586.6	599.35	825.3	687.5	1202.4
Fresh weight (gm.)	0.1	0.087	0.101	0.132	0.16	0.148	0.166	0.122	0.126	0.0875	0.11
Dry weight (gm.)	0.022	0.0255	0.0187	0.0239	0.0216	0.0237	0.026	0.0239	0.0207	0.0058	0.0187

Table 8.

Effect of crude and diluted form of Kalmegh seed extract on seedling behavior of Moong

Parameters	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Root length (cm.)	9.8	5	6.375	8.7	8.8	8.4	8.5	9.84	10.8	9.4	11.6
Shoot length (cm.)	10.2	7.83	13.25	11.2	11.6	10.2	12	9.1	11.2	12.1	11.16
Seed vigor	2000	641.5	1962.5	1990	2040	1860	2050	1894	2200	2150	2276
Fresh weight (gm.)	0.4034	0.226	0.406	0.3534	0.4304	0.385	0.4465	0.438	0.4034	0.342	0.392
Dry weight (gm.)	0.03	0.0166	0.027	0.022	0.0308	0.03	0.0325	0.032	0.032	0.026	0.028

increased almost three times more in T6 and T10; shoot lengths remained unaffected rather decreased very much in T8; seed vigor decreased mostly but positive increase in T5, T8 and T10.

The above results suggest specific dilution treatments can be effectively practiced to get better results in case of above crop agriculture.

Table 9.

Effect of crude and diluted form of Kalmegh seed extract on seedling behavior of Lentil

Parameters	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Root length (cm.)	8.86	0	9.36	9.1	10.6	11	11.4	10.64	9.09	11.6	11.9
Shoot length (cm.)	8.4	0	8	9.625	8.9	9.5	9.8	8.2	7.78	9.2	8.8
Seed vigor	863	0	1041.6	1310.7	1560	1230	424	1318	843.5	1248	1656
Fresh weight (gm.)	0.0706	0	0.1	0.1255	0.1146	0.1	0.14	0.143	0.123	0.1288	0.135
Dry weight (gm.)	0.012	0	0.0128	0.0135	0.012	0.0125	0.0144	0.012	0.011	0.0134	0.0132

Table 10.

Effect of crude and diluted form of Kalmegh seed extract on seedling behavior of Gram

Parameters	TREATMENT CONDITIONS										
	control	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Root length (cm.)	6.33	0	20	16.37	12.58	16.8	17.16	15.9	14.6	16.1	17.2
Shoot length (cm.)	14.4	0	12	12.5	12.2	15	12.33	10.4	10.2	12.5	13.2
Seed vigor	1658.4	0	640	1154.8	1734.6	2226	1179.6	1841	744	572	2128
Fresh weight (gm.)	0.35	0	0.36	0.69	0.67	0.74	0.75	0.76	0.7	0.82	0.884
Dry weight (gm.)	0.1193	0	0.18	0.1	0.108	0.1059	0.119	0.106	0.102	0.107	0.11

REFERENCES

Abdul – Baki, A.A., and J.D., Anderson,1973. Vigor determination in soybean by multiple criteria. Crop Sci., **13**: 630-633.

Kapil, A., I.B. Koul, S.K. Banerjee, and B.D. Gupta. Antihepatotoxic effects of major diterpenoid constituents of*Andrographis paniculata. Biochemical Pharmacology* **46**(1):182-85. 1993

Kumar R. N., Chakraborty S., Kumar N., Method to break seed dormancy of Andrographis Paniculata.Advance in Bioresearch, **1**(2) 139-143, December 2010.

Kundu S., Pradhan B., Santra A., Dutta C. A. and Kundagrami S. Effect of herbal seed treatment and Hogland nutrient solution on improving vigour and productivity of rice (*Oryza sativa*). Journal of Botanical Society of Bengal **67**(1): 29- 36. 2013.

Ma L., Wu H., Bai R. Zhuo L., Yuan X., Hou D. Phytotoxic effects of *Stellera chamaejasme* L. extract. African Journal of Agricultural research **6**(5), 1170- 1176. 2011.

Nair G.S., Sudhadevi P.K., Kuria A. Introduction of medicinal & Aromatic Plants as Intercrops in coconut Plantation. Recent advances in Medicinal,Aromatic & Spice crops,vol **1**, 3-18, 163-165, 1991.

Puri, A., R. Saxena, R.P. Saxena, and K.C. Saxena. Immunostimulant agents from *Andrographis paniculata*. J. Natural Products **56**(7): 995-99, 1993.

Roychowdhury D., Santra A., Barman J.K., Das M and Kundagrami S. Effect of Seed Treatmen on Improving vigor and Productivity of Lentil *(Lens culinaris)* in Heat Stressed condition. Indian Biologist, **45**(2), 65 – 72, 2013.

Varma A., Padh H. Shirivastanava N.; Andrographolide : A new plant derived antineoplastic entity on Horzon, Oxford Journals, 2009.

Yaseen M, Tripathi R S,Integration of medicinal and aromatic crops with food crop, CIMAP Annual Report, 25, 2009.

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STUDIES ON CHANGES IN CHLOROPHYLL CONTENTS IN LEAVES OF TREE FRUITS

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ABSTRACT

An experiment was carried out during 1989-90 on chemical analysis for determining the total chlorophyll content and its two fractions chlorophyll a & chlorophyll b in leaves of four commercial varieties of mango namely Langra, Krishnbhog, Himsagar, Fazli and a wild variety Darjeeling wild. Leaves were collected from mature mango trees at two stages of leaf growth-immature and mature. In a mature stage, total chlorophyll was maximum (4.123 mg/g of fresh weight) in the variety Darjeeling wild and minimum in Fazli variety (1.965 mg/g of fresh weight). In respect of two fractions of chlorophyll a was highest in the Darjeeling wild (2.122 mg/g of fresh weight) and the lowest in the variety Fazli (1.071 mg/g of fresh weight). This pattern was maintained with the other fractions of chlorophyll though the level of this pigment was slightly less than that of the chlorophyll a in each variety. In the immature leaf in all the varieties, the levels of the chlorophylls were much less than those noted in the mature leaves. However, the relative proportions of chlorophyll were higher than those of chlorophyll a in all cases.

INTRODUCTION

Wonder of the organic world is the strapping of solar energy and its conversion into carbon compounds. In this vital process the chlorophylls performed the most critical part. in plant kingdom a number of different kinds of chlorophyll occur. In the higher plants these pigments occur predominately in leaf tissues. In this tissue chlorophyll a is almost universally present. Another type of chlorophyll pigment chlorophyll-b- is also found widely in plant leaves. Both these types of chlorophyll have many characteristics properly by which they are generally identified.

Number of factors can affect chlorophyll synthesis. More important of these factors are genetic, light, oxygen supply, optimum temperature level and supply of certain key elements like nitrogen, magnesium and iron etc.

In India mango occupies the highest position among the fruits. It has been reported that annual production of this fruit is about 9.2 m. tones (Rao, 1989).

Because of its great importance this fruit is widely grown in different parts of India. However variability of agro climatic conditions have given rise to numerous local varieties suited to different agro climatic conditions. In eastern India also there are more than choice varieties in cultivation. The present study covers five of such varieties namely Langra, Kishanbhog, Himsagar, Fazli and Darjeeling wild.

One the special feature of most of the commercial varieties of mango is that they are prone to irregular bearing habits. One of the possible reason is commonly put forward in explaining such bearing characteristics is that during an "on" year mango tress become exhausted of their food reserve. Since functions of chlorophylls are directly related to carbohydrate synthesis there may be a possibility that different varieties may show variations between their respective chlorophyll contents. The study under report was intended to verify this possibility in four commercially important varieties of this fruit namely Langra, Kishanbhog, Himsagar and Fazli. In addition to these and wild variety collected from Darjeeling district has also been included in this study. Since age of the leaf may affect chemical constituent of leaves, samples were collected at two different stages of leaves, immature and mature and these were used for determining their chlorophyll contents. The results of these analyses have been presented and discussed upon herewith.

MATERIALS AND METHODS

The present experiment was conducted at Baruipur Agricultural Farm, Calcutta University, 25 km away from University College of Agriculture. The investigation was done on mango varieties which were about 10 years of age. The chlorophyll estimation was performed in the month of May from the immature and mature leaves of different mango varieties.

Method of Estimation

(i) Estimation of chlorophyll in immature leaf :

In all the cases pigment were collected from fresh material i.e. the loaf, considering a single variety of mango at each time. At first disc shaped circular portion of the leaf was prepared by punching with the help of punching instrument. Then the circular punched portion of the leaves were crushed with the help of mortar and pastle addition 1 : 1 methanol and ethanol mixture and the volume was made 10 ml. Now filtration was done using Whatman filter paper No.1. Took 2 ml filtrate and was diluted to 10 ml by addition 1:1 methanol and ethanol mixture and colorimeter reading was recorded with the help of Spectrophotometer for the above extract directly in 645 and 663 nm. Following the above method one set of blank solution was also prepared in absence of chlorophyll.

Cha = (12.7 x D 663 – 2.69 x D 645) x dilution factor (.1)

Chb = (22.9 x D 645 – 4.68 x D 663) x dilution factor (.1)

(ii) Estimation of chlorophyll in mature leaf :

From fresh mango leaves of separate many varieties pigment were extracted. Each sample was first cut into the shape of a circular disc employing a punching instrument. Then the circular punched portion of the

Table

CHLOROPHYLL CONTENT (mg/g OF FRESH WEIGHT) OF VARIOUS MANGO VARIETIES ON THE MONTH OF MAY

Varieties	Chlorophyll a and b	Immature leaf mg/g of fresh weight	Ratio of Cha and Chb	Mature leaf of fresh weight	Ratio of Cha and Chb	Ratio of total Chlorophyll of immature and mature leaf
Langa	Ch a	0.203		1.874		
	Ch b	0.231	0.874	1.574	1.190	0.125
	Total Chlorophyll a & b	0.434		3.448		
Kishanbhog	Ch a	0.061		1.519		
	Ch b	0.779	0.078	1.22	1.353	0.318
	Total Chlorophyll a & b	0.840		2.641		
Himsagar	Ch a	0.169		2.142		
	Ch b	0.213	0.793	1.813	1.818	0.096
	Total Chlorophyll a & b	0.382		3.955		
Fazli	Ch a	0.006		1.071		
	Ch b	1.056	0.005	0.894	1.197	0.540
	Total Chlorophyll a & b	1.062		1.965		
Darjeeling wild	Ch a	0.294		2.122		
	Ch b	0.452	0.650	2.001	1.060	0.180
	Total Chlorophyll a & b	0.746		4.123		

leaves were crushed with the help of mortar and pastle addition 1:1 methanol and ethanol mixture and the volume was made 10 ml. Now filtration was done using whatman filter paper No.1. Took 2 ml filtrate and was diluted to 30 ml by adding 1:1 methanol and ethanol mixture and colorimeter reading was recorded with the help of Spectrophotometer for the above extract directly in 645 and 663 nm. Following the above method, one set of blank solution was also prepared in absence of chlorophyll.

Cha = (12.7 x D 663 – 2.69 x D 645) x dilution factor (.3)

Chb = (22.9 x D 645 – 4.68 x D 663) x dilution factor (.3)

RESULTS AND DISCUSSION

Data in the table will show that (a) variability of chlorophyll contents between an immature and a mature leaf within a variety and (b) variability between varieties at both the stages of leaves. However in all the varieties chlorophyll a in immature leaf was less than that of the chlorophyll b. In the mature stage both these fractions of chlorophyll showed manifold increased in all the varieties.

The trend of changes in the respective levels of chlorophyll a and chlorophyll b showed some notable aspects. Among all the varieties in the young leaves chlorophyll a lowest in Fazli and highest in the variety Darjeeling wild. The variety Fazli also showed highest content chlorophyll b against the lowest content in the variety Himsagar. When the ratios of the two pigment fractions were compared (Chlorophyll a / Chlorophyll b) the highest level was noted with the variety Langra and the lowest level was noted in case of variety Fazli.

In case of the mature leaves highest amount of chlorophyll was noted in case of Darjeeling wild and lowest amount in the variety Fazli. It can be seen from the table that when the leaves were immature highest content chlorophyll was noted in this variety. it appears that in the Fazli variety chlorophyll content do not mark so much increase as it could be noted in case of any other variety. It is also evident from the table that among the different varieties the magnitude of increase in total chlorophyll content was highest in the variety Langra. Surprisingly though the increase in absolute amount was maximum in Darjeeling wild it was much less than that either in the variety Himsagar or the variety Langra.

In the mature stage of leaves the ratio of chlorophyll a and chlorophyll b was the lowest in case of Darjeeling wild and it was highest in case of Kishanbhog. However these ratios in two other varieties Langra and Fazli were relatively closer to that of the Kishanbhog.

REFERENCES

Anderson, T.W. (1984). An introduction to multivarieties Statistical Analysis. John Wiley & Sons. New York, Chap. 6. pp. 195-243

Bakshi, J.C. and Bajwa, B.S. (1957). Identification of some mango varieties in the nursery stage. The Indian Journal of Horticulture Sc.vol.**14**(1), pp. 47-52.

Madhava Rao, V.N. (1989). The Hindu, Survey of Indian Agriculture, M/s. Kasturi & Sons Ltd., National Press, Madras.

Mukherjee, S.K. (1948). The varieties of mango (Mangifera indica L.) & their classification. Bull. 2, Bot. Soc. Beng.

Naik, K.C. and Ganguly, S.R. (1950). A monograph on classification and nomenclature of South India mangoes. PP. 312-318. Superintendent Government Press, Madras.

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STUDIES ON VERMICOMPOST OF WATER HYACINTH AND COWDUNG WITH *Eisenia fetida*

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ABSTRACT

Composting/ vermicomposting are an efficient way of organic waste disposal together with preserving nutrients for crop production. Quality of vermicompost of water hyacinth along with cow dung using *Eisenia fetida* was determined. Quality parameters included stability and maturity of the product, free living aerobic diazotrophic population and dinitrogen fixation. Data indicated that the vermicompost of water hyacinth promises to be a good choice organic supplement for crop production.

Keywords: Stability and maturity of vermicmpost, Water hyacinth, Free living diazotrophic bacteria, dinitrogen fixation.

INTRODUCTION

An amount of 38 billion metric tons organic waste is produced worldwide (Sutha, 2009). Environmental friendly and safe disposal of those waste is a global concern. The water hyacinth (*Eichhornia crassipes*), a native aquatic weed of brazil and other central south American countries (Center, 1994) is an invasive aquatic macrophyte with a particular potential for rapid growth and dispersal (Cook, 1990). Water hyacinth causes major problems in many irrigation systems and because of the high cost of removal and its rapid growth this mostly remains unharvested and unutilized. One of the easiest ways to utilize water hyacinth is composting. The process of vermicomposting is faster than composting. The product is rich in microbial activity and plant growth regulators, and fortified with pest repellent attributes as well. It is also a good source of plant nutrients, have plant growth promoting activity, improve crop growth and yield. It reduces the C:N ratio and retains more N than the traditional methods of preparing compost (Gandhi *et al*., 1997).

The quality of a good compost including vermicompost depends on its stability and maturity. Application of unstable or immature compost may inhibit seed germination, reduce plant growth and damage crops by competing for oxygen or causing phytotoxicity to plants due to insufficient biodegradation of organic matter (Wu *et al*., 2000; Brewer and Sullivan, 2003 and Cooperband *et al*., 2003).

Several reports are available on enumeration of different microbial population in vermicompost with different bedding materials and earthworms (Mageswari and Sudha, 2013; Selvi, 2013). But information regarding aerobic free living diazotrophic bacterial population and di-nitrogen fixation in vermicompost of water hyacinth and cowdung using *Eisenia fetida* is scarce. Present effort was initiated to enumerate aerobic free living diazotrophic bacterial population and di-nitrogen fixation in vermicompost of water hyacinth. Stability and maturity of this compost was also examined by respiration and seed germination test respectively.

MATERIAL AND METHODS

Collection of water hyacinth and vermicomposting

Water hyacinth (*Eichhornia crassipes*) were collected from several ponds and ditches situated near the Agricultural Experimental Farm (22°22′ N and 86°26′ E) of the Calcutta University, Baruipur, south 24 Parganas. Collected material were chopped into small pieces and allowed for partial decomposition for 15days.

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Urine free cowdung was obtained from farmer's cattle shade situated near the Farm. Then the partial decomposed material was mixed with cowdung in 3:1 ratio. Vermicomposting pits were prepared by following methods: Pits of 0.75 x 0.75 x 0.25m^3 sizes were made under covered shade at the Farm and was filled with mixture of partial decomposed water hyacinth and cowdung. The earthworm *Eisenia fetida* as used in this experiment was taken from the stock of the Agricultural Experimental Farm, University of Calcutta, Baruipur. Healthy, juvenile earthworms collected from the stock culture were released on the bed at the rate of 20 earthworms/square feet. Regular watering was also done as and when required with equal amount in each bed. Four different pits were chosen for this experiment.

Sampling of vermicompost

Four replicated samples from each of four pits (WH-1, WH-2, WH-3 and WH-4) were collected for laboratory analysis at every 15 days interval up to 75 days. It was observed that the composting was completed at 75 days. Only the data on the samples on 75 days are presented herein.

Analytical procedure

Physico-chemical properties of the vermicompost samples were determined by standard methods (Jackson, 1975). Enumeration of aerobic non-symbiotic diazotrophic bacteria was done by compost dilution plate technique using LG agar culture medium (Dobereiner, 1995). The results were expressed as log colony forming unit. Estimation of N_2 fixation in vermicompost was done by macro-Kjeldahl method (Allen, 1957). Stability study of vermicompost was done by respiration (Alef, 1995) test. The maturity of the vermicompost was done by seed germination test (Helfrich *et al.,* 1998) followed by calculation of germination index (GI).

Statistical analysis

Assigning different lots of vermicompost as treatment factor, analysis of variance (ANOVA) was carried out by Completely Randomized Design (CRD) using SPSS 11.0 statistical package. The factor vermicompost lot had four levels and the replicate had four levels. The least significance difference (LSD) test was applied to evaluate the significance of difference between individual treatment factors. The treatment means were compared by Duncan's multiple range test at 0.05P.

RESULT AND DISCUSSION

The pH and the electrical conductivity of vermicompost in different lots did not varied significantly among themselves (Table 1). Except WH-3 the organic carbon content of the vermicompost WH-1, WH-2 and WH-4 was statistically similar. Total nitrogen content of the vermicompost samples followed the trend of their organic carbon content. The C/N ratio was varied from 7.79 to 8.14, which values were very narrow. Narrower C/N ratio indicating greater decomposition of the waste material as well as better quality of the compost (Gaur and Singh, 1995).

The population of diazotrophic microorganisms, dinitrogen fixation of the bacteria present in the Vermicompost and compost respiration rate in different lots varied significantly (Table 2). The highest nitrogen fixation bacterial population was recorded in WH-3 (7.33) which was related to its N_2 fixating ability (2.4 mg nitrogen fixed 50 mL^{-1} culture medium). The other lots could be ranked as WH-4>WH-1>WH-2. Statistically similar compost respiration rate was recorded in WH-1, WH-3 and WH-4 lots of vermicompost. The highest result was recorded in WH-4 (0.1125 mg CO_2-C gm^{-1} vermicompost C day^{-1}) compost. According to Epstein (1997) compost having respiration rate < 2 mg CO_2-C gm^{-1} vermicompost C day^{-1} is very stable one. In our study all lots of vermicompost registered much lower respiration rate than the standard value, indicating that the different lots of vermicompost under study were very stable.

The germination index (GI) (Table 3) value of rice varied between 73.59 to 77.77 %, and that of wheat, pea and gram were 75.47 to 77.28 %, 81.27 to 87.26 %, and

Table 1.

Physico-chemical Properties of vermicompost under study

Vermicompost code	pH (1:2.5)	Electrical conductivity (dS m^{-1})	Organic carbon (gkg^{-1})	Total nitrogen (gkg^{-1})	C:N Ratio
WH-1	7.20 [a*]	2.33 [a]	82.7 [a]	10.4a	7.95a
WH-2	6.97 [a]	2.37 [a]	86.5 [a]	11.1a	7.79a
WH-3	6.80 [a]	2.25 [a]	75.0 [b]	9.5b	7.89a
WH-4	7.27 [a]	2.06 [a]	82.2 [a]	10.1a	8.14a

*Figures denoted by same alphabets are statistically similar at 5% Probability level by DMRT

Table 2.

Nitrogen fixing population, N_2 fixation and respiration rate of vermicompost under study

Vermicompost code	Population of N_2 fixing microorganisms (log colony forming unit)	Dinitrogen fixation (mg nitrogen fixed 50 mL^{-1} culture media)	Respiration (mg CO_2-C gm^{-1} vermicompost C day^{-1})
WH-1	7.28 [b*]	2.14 [b]	0.0755[b]
WH-2	7.23 [c]	1.91 [c]	0.1013[a]
WH-3	7.33 [a]	2.40 [a]	0.1093[a]
WH-4	7.29 [b]	2.19 [b]	0.1125[a]

*Figures denoted by same alphabets are statistically similar at 5% Probability level by DMRT

Table 3.

Germination index (GI) value in case of four seed material

Vermicompost code	Rice (%)	Wheat (%)	Pea (%)	Gram (%)
WH-1	73.59[a*]	77.28[a]	82.97[ab]	93.76[ab]
WH-2	75.06[a]	75.47[a]	81.27[b]	92.32[bc]
WH-3	75.45[a]	76.31[a]	82.98[ab]	90.17[c]
WH-4	77.77[a]	76.91[a]	87.26[a]	95.29[a]

*Figures denoted by same alphabets are statistically similar at 5% Probability level by DMRT

90.17 to 95.29 % respectively. According to Helfrich *et al.* (1998) a GI value ≥ 70 % indicates a lower level of phytotoxic substances in the compost. In this present study with four different seeds all the GI values were above 70%, which indicate the presence of low phytotoxic substances in the vermicompost. Hence different lots of vermicompost under study were mature.

CONCLUSION

Quality vermicompost could be prepared from water hyacinth, cow dung and *Eisenia fetida.* Because it harbouring sufficient number of nitrogen fixing microorganisms with good nitrogen fixing ability. This vermicompost will enrich fertility of land and soil.

REFERENCES

Alef, K. (1995). Soil respiration. In: Methods in soil microbiology and biochemistry. Alef, K. and Nannipieri, P. (eds.). Academic Press, London, UK. pp. 214-219.

Allen,O.N. (1957). Experiments in soil bacteriology, Burgess Publishing Co., Minneapolis, Minnesota. 83-86.

Brewer, L. J. and Sullivan, D. M. (2003). Maturity and Stability Evaluation of Composted Yard Trimmings. Compost science and utilization. **11**(2): 96-112.

Center, T.D. (1994). Biological control of weeds : Waterhyacinth and water letter. In: Pest Management in the subtropics: Biological Control – A Florida Perspective. Rosen, D., Bennett, F.D. and Capinera, J.L. (eds.). Intercept Publishing Company, UK, pp. 481-521.

Cook, C.D. (1990). Origin, Autoecology and spread of some of the world's most troublesome aquatic weeds. In: Aquatic weeds: the ecology and management of nuisance aquatic vegetation. Pieterse, A.H. and Murphy, K.J. (eds.). Oxford University Press, UK. PP. 612.

Cooperband, L.R.; Stone, A.G.; Fryda, M.R. and Ravet, J.L. (2003). Relating compost measures of stability and maturity to plant growth. Compost Science and Utilization. **11**: 113–124.

Dobereiner, J. (1995). Isolation and identification of acrobic nitrogen fixing bacteria from soil and plant. In: Methods in applied soil microbiology and biochemistry. Alef, K. and Nannipieri, P. (eds.) Academic Press, London, UK. PP. 134-135.

Epstein, E. (1997). The science of Composting. Technomic Publishing Company, USA, PP. 487.

Gandhi, M.; Sangwan,V.; Kapoor, K.K. and Dilbaghi, N. (1997). Composting of household wastes with and without earthworms. Environment and Ecology. **15**(2): 432-434.

Gaur, A.C. and Singh, G. (1995). Recycling of rural and urban wastes through conventional and vermin composting. In: Recycling of crop, animal, human and industrial wastes in agriculture. Tandon, H.L.S (ed.). Fertilizer Development and Consultation Organisation, New Delhi, India. pp31-49.

Helfrich, P.; Chefetz, B.; Hadar, Y.; Chen, Y. and Schnabl, H. (1998). A nobel method for determining phytotoxicity in compost. Compost Science and Utilization. **6**(3): 6-13.

Jackson, M.L. (1975). Soil Chemical analysis. Prantic Hall of India, New Delhi.

Maheswari, N. Uma. And Sudha, S. (2013). Enumeration and detection of phosphate solubilizing bacteria from the gut of earthworm varities. Journal of Chemical and Pharmaceutical Research. **5**(4): 264-267.

Selvi, B. K. (2013). Preparation of phosphate solubilizing microbial inoculants using vermicompost as a carrier material. Research Journal of Agricultural Science. **4**(2): 146-149.

Suthar, S. (2009). Vermicomposting of vegetable – market solid waste using *Eisenia fetida*: Impact of bulking material on earthworm growth and decomposition rate. Ecological Engineering. **35**: 914-920.

Wu, L.; Ma, L.Q. and Martinez, G.A. (2000). Comparison of methods for evaluating stability and maturity of biosolids compost. Journal of Environmental Quality. **29**: 424-429.

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VARIABILITY OF HOLOCELLULOSE AND LIGNIN COMPONENTS IN SOUND AND DECAYED WOOD OF TEAK (*Tectona grandis*)

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ABSTRACT

Different methods have been suggested by various workers (Norman and Jenkins, 1933; Wise, Murphy and D' Addico, 1946; Jhonston, 1962 and Maitra, 1991) for the determination of holocellulose, lignin and extractive free contents of wood, together with true cellulose and hemicellulose.

The test blocks (normal, partially decayed and wood decayed in nature) selected for chemical analysis have been grounded separately in Willey mill to pass 40 mesh standard size for the beter penetration of the solvents and chemical regents. Cowling (1960) has suggested that the size of the particles should be same because this influence the different processes like determination of alkali solubility and holocellulose contents in this study. The samples have been stored in air tight glass container and mixed thoroughly to randomise before each aliquot part has been taken for chemical analysis.

Wood-destroying basidiomycetes normally occur in nature as dikaryotic mycelia and almost all laboratory investigations conducted on or with them use dikaryotic mycelial cultures. A few attempts appear to have been made to compare the physiological behavior of monokaryotic mycelia with that of related dikaryotic mycelia. The findings became of considerable interest to us following the discovery that, in the presence of wood preservatives and other fungitoxic agents, dikaryotic mycelia of wood destroying basidiomycetes often reverted to the monokaryotic conditions.

INTRODUCTION

Cowling (1960) has modified the method of TAPPI standard T 12m – 45 for this analysis. Cowling's (1960) modified method has been followed in the present investigation for the analysis of total extractive materials from sound, partially decayed and naturally decayed wood.

Cowling (1960), however, has followed procedure as in TAPPI standard T 12m-45, for the determination of holocellulose in wood and this has been followed in the present study.

MATERIALS AND METHODS

0.5(± .0lmg) of samples of air-dry wood meal of known moisture contents have been rendered extractive and moisture-free according to the procedure followed by Cowling (1960). Each test sample has then been taken in a sintered glass crucible (IG 3) and chlorinated within ice cold jacket for 5 minutes with moderate suction in a chlorination apparatus designed by Cowling (1960). The chlorinated sample has been covered with 25 ml of ethanol for 1 minute and washed with distilled water. The sample is subjected to two treatments with 3% ethanolic monoethanolamine solution (by volume) at 75 -80°C for two minutes each following washing with 25ml ethanol twice and with ice-cold distilled water twice. This whole process is repeated twice. At the time of addition of each solvent during washing of monoethanolamine, the samples are thoroughly stirred by neutral glass rod and at each time solvents are removed by suction. To obtain essential white colour (showing no colour change when treated with hot monoethanolamine) of the samples, the process of chlorination has been performed for 8-10 times. Finally, each crucible containing sample is washed with 25 ml of ethanol and with 25 ml of ethyl ether thrice. Then the crucibles are dried in an oven at 35ºC for two hours, finally dried to constant weight in a vacuum over magnesium perchlorate and then reweighed. The yield of holocellulose is calculated as a percent of the weight of the original moisture-free sample in each case.

Alpha, Beta and Gamma-cellulose in Holocellulose prepared from sound and decayed wood :

Holocellulose contents in wood is composed of two fractions, viz. alphacellulose or true cellulose and hemicelluloses containing beta and gamma - celluloses. Cowling (1960) has stated that these three basic fractions of holocellulose differ mainly in the number and type of sugar units that comprise their individual molecules. During decay of wood, these cellulose molecules are used up as the main food by the fungal mycelia. Already prepared holocellulose sample is transferred to a 250 ml beaker, to this 25 ml of 17.5% sodium hydroxide solution (by weight) is added. The beaker is then kept in water bath at 20° (±0.5°) C for 5 minutes, and the flattened end of neutral glass rod is used to stir the mixture for thorough maceration. After sometime 25 ml of 17.5% sodium hydroxide solution is added to it and the whole set up is kept in the water bath for 45 minutes. 50ml of distilled water is then added with thorough stirring for maceration, then filtered through a sintered glass crucible (IG 3) and washed with distilled water. 25 ml of 10% acetic acid is added to each sample and kept at room temperature for 5 minutes and lastly washed with 25 ml of distilled water each time until the sample in each crucible is rendered acid free. The filtrate is collected in a flask for determination of beta and gamma-celluloses. The crucible- containing alpha-cellulose only is washed twice with 25 ml of ethanol each time and thrice, with 25 ml of ethyl ether each time. The crucible is then wiped clean with ether-moistened cellulose-tissue, air dried in an oven at 35° (±0.5°)C, dried to constant weight in a vacuum over magnesium perchlorate and finally weighed.

The filtrate of each sample containing beta and gamma cellulose is found to be alkaline in nature. To make every sample acidic, 20 ml of glacial acetic acid is added to each filtrate and kept for 48 hours for precipitation of beta-cellulose. The precipitate thus obtained is filtered through a sintered glass crucible (IG 5), washed twice with 25 ml of distilled water, air dried, dried to constant weight in a vacuum over magnesium perchlorate and finally reweighed.

According to the formulae of Cowling (1960), the yield of alpha and beta cellulose in each sample has been expressed in percentage. By subtracting the percentage of alpha and beta-cellulose from the total percentage of holocellulose, the yield of gamma cellulose has been determined in each sample.

The results obtained during the experimental period are given in Table 1 & 2 for every sample on mean weight based on readings.

Lignin in Sound and Decayed Wood :

Following Cowling (1960), the determination of Lignin in sound, partially and naturally decayed wood has been estimated. The lignin is condensed to an insoluble residue and this is determined gravimetrically. Samples of air dried wood meal of known moisture content is taken in several 30 ml. shell vials and chilled in ice cold water 3 ml of ice-cold 72.01 percent sulphuric acid (w/v) is added to each sample. During ice treatment, the sample is macerated with the flat end of a natural glass rod for 3 minutes. Then the vials are fastened to shakers in a water bath at 30° (± 0.5) C. Every 15 minutes, the shaker is stopped and to ensure complete penetration of the acid into the particles, the samples are grounded against the inner wall of the vial with a flattened end of a neutral glass rod. The content of each vial is removed to a 250 ml beaker after one hour. The vial is washed with 84 ml of distilled water in small aliquots to dilute the concentration to 4 percent weight. Then the each set is covered with a water glass and autoclaved at 121° (± 1)C for one hour.

The suspension of the sample is filtered through a sintered glass crucible (IG3) after cooling and washed several times to render the residue acid- free, air dried, dried to constant weight at 105° (± 1)C, and finally reweighed. The lignin residual weight is expressed in percentage of moisture free weight of the original sample. The result obtained during the experimental period are given table 1 & 2.

Data indicated in the Table 1 & 2 show that both the monokaryotic and dikaryotic mycelia utilize lignin during its growth within the wood. It further reveals that the monokaryotic mycelium appears to be less virulent than the dikaryotic one.

Moisture content in sound and decayed wood :

Following the method in Cowling (1960), the moisture contents in sound and decayed wood have been determined. 1 gm (± 0.1 mg) of the test samples to be analyzed was taken out from the container and weighed in a tarred weighing bottle. Ten such weighing bottles in replicates of three have been placed in a vacuum desiccator containing magnesium perchlorate under reduced pressure of 3 mm. of mercury for at least 12 hours and the lids of the bottles were removed in the desiccator before the experiment was started. After 12 hours of the experiments, the vacuum was released

slowly through a tube containing magnesium perchlorate. The desiccator was then opened, the lids replaced on the weighing bottles as quickly as possible and these have then been weighed. The whole process has been repeated to obtain a constant weight in every case. The result obtained with every sample has been expressed on average readings. The results are given in Table 1-2.

The result shows that the moisture contents of the wood gradually increased in the decayed woods which have been infected by both monokaryotic and dikaryotic mycelia.

Total extractive in sound and decayed wood :

0.5 gm (+0.01 mg) samples of air-dry wood meal of known moisture content have been subjected to extraction in a Soxhlet's apparatus successively with the mixture of 95 percent ethanol and grade benzene in the ratio of 1:2 by volume and then with ethanol alone for 8 hours. The Soxhlet is siphoned approximately three times per hour and in this way these extractions have been regulated. The excess solvent in the test samples after extraction period has been removed by suction. The solvent in the extraction flasks has been evaporated and dried to constant weight at 105°C. The increase in weight in each flask indicates the ethanol-benzen and ethanol solubility of the sample, expressed as a percentage of moisture free weight.

The sample has been extracted with hot water by washing it quantitatively into 1 liter beaker with 750 ml. of distilled water for all types of wood. The beakers containing water suspensions have then been simmered on a hot plate at 92°C. for 3 hours. The extractive free samples have then been filtered through sintered glass crucibles (IG 3) washed with hot distilled water quantitatively. Then the aliquot parts have been air dried,

Table 1.

Data (mean) showing the percentage of holocellulose components in sound and experimentally decayed sapwood of Teak (*Tectone grandis*) by the monokaryotic mycelia of *Polyporus grammocephalus* Berk under different incubation periods.

Name of the wood components	Nature of Wood					
	Sound Wood	Experimentally decayed wood				Naturally decayed wood
		Monokaryotic (AB) mycelia (DAYS)				
		30	60	90	120	
Moisture Content	12.60	13.00	14.14	14.10	14.00	13.90
Holocellulose content	60.00	52.02	49.12	47.04	45.24	37.78
i) α-Cellulose	38.50	31.16	29.00	28.00	27.00	21.76
ii) β-Cellulose	6.26	6.06	5.76	5.24	5.00	4.68
iii) γ-Cellulose	15.24	14.80	14.36	13.80	13.24	11.34
Lignin content	26.26	25.96	24.64	24.76	22.36	14.86

Table 2.

Data (mean) showing the percentage of holocellulose components in sound and experimentally decayed sapwood of Teak (*Tectona grandis*) by the dikaryotic mycelia of *Polyporus grammocephalus* Berk under different incubation periods

Name of the wood components	Nature of Wood					
	Sound Wood	Experimentally decayed wood				Naturally decayed wood
		Monokaryotic (AB+ab) mycelia (DAYS)				
		30	60	90	120	
Moisture Content	12.68	12.90	13.31	13.62	13.88	14.20
Holocellulose content	59.80	49.36	47.26	45.63	43.06	37.78
i) α-Cellulose	38.26	29.00	27.62	26.78	25.10	21.76
ii) β-Cellulose	6.26	6.00	5.56	5.18	4.96	4.68
iii) γ-Cellulose	15.24	14.36	14.08	13.67	13.00	11.34
Lignin content	26.26	26.00	25.10	24.23	23.10	14.86

dried to constant weights in a vacuum over magnesium perchlorate, and finally weighed. The amount of total extractive materials present in the different samples have been calculated by the difference in weight between the extractive free and the original wood meals.

RESULTS AND DISCUSSIONS

It is evident from the Table that the amount of total extractive materials present in the decayed wood samples have been found to be less in every case than those in sound wood. It has also been proved that the extractives in normal wood and the degraded products of the wood components undergo gradual depletion due to fungal decay.

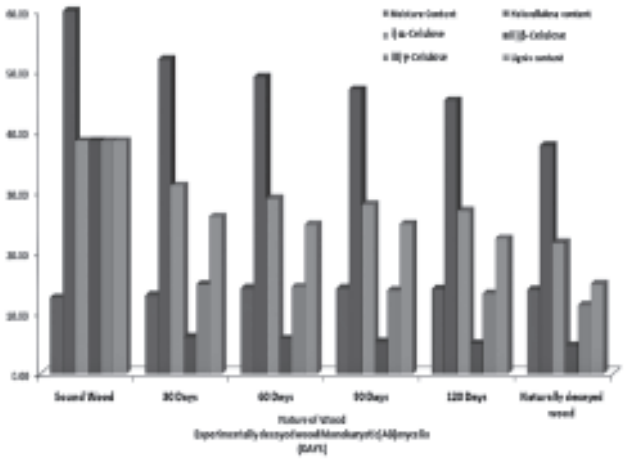

Fig. 1. Graph showing the percentage of holocellulose components in sound and experimentally decayed sapwood of Teak (*Tectone grandis*)) by the monokaryotic mycelia of *Polyporus grammocephalus* Berk. under different incubation periods

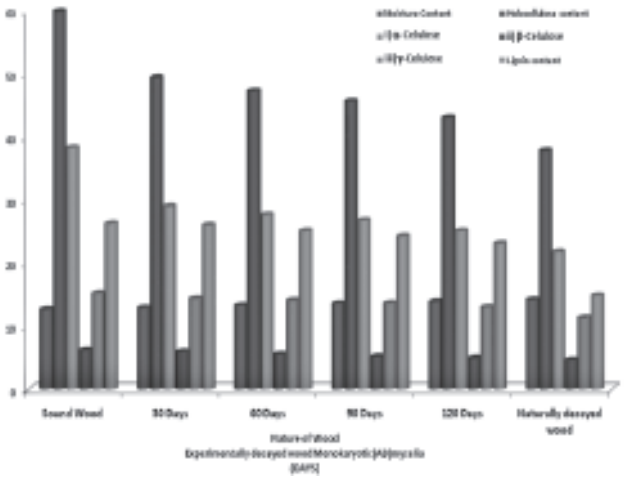

Fig. 2. Graph showing the percentage of holocellulose components in sound and experimentally decayed sapwood of Teak (*Tectona grandis*) by the dikaryotic mycelia of *Polyporus grammocephalus* Berk. under different incubation periods

The results from the Table 1 & 2 shows that both the monokaryotic and dikaryotic mycelia of *Polyporus grammocephalus* Berk. utilize total cellulosic contents considerably and hence degradation in woody tissues take place.

The findings of the present investigation on the decaying capabilities of wood of Teak (*Tectone grandies*) by four monokaryotic (AB, ab, Ab and aB) and two dikaryotic (AB+ab; Ab +aB) mycelia of the wood rotting fungi namely *Polyporus grammocephalus* Beak. are as follows :

The cellular and lignin component of the Teak wood are found to be decomposed by the monokaryotic & dikaryotic mycelia of the test-fungi were very much prominent.

The data on the lignin decomposition have revealed that it is a relatively slow process and conditioned by some critical cultural parameters in cases of the test fungi. These type of lignin decomposition might be the same for all the white rot fungi in general (Kirk *et al.*,1973).

The experimental findings further revealed that the ligninolytic activities leading to wood decaying capacity and lignin decomposition of the test fungi are genetically controlled possibly at a mutagenic level. These genes are possibly arranged in close sequence and are not separated during genetic exchange and consequent segregation to off-springs from parents. The increased activities in the dikaryotic mycelia are due to the presence of two sets of such gene sequence ie. double dosing effect. Such double doses lead to accelerated action upto a certain level due to gene interaction. It was assumed that the decaying capabilities and ligninolytic enzyme system were genetically controlled and the same was segregated in the Mendelian segregation type.

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REFERENCES

Cowling, E.B. (1960) Methods for chemical analysis of decay wood. U.S. For. Serv. Prod. Lab. Tech. Bull. No. 2177.

Jhonston, H.H. (1962) The decomposition of Cellulose by soil fungi. Ohio J SCI., **62**: 108-112. cited in Rev. appl. Mycol., **42**: 2.

Kirk, T.K. and Highley, T.L. 1973. Quantitative changes in structural components of conifer woods during decay by white and brown rot funji. Phytopathology, **63**(ii): 1338-1342, USDA, Madisom, Wis.

Maitra , T.K. (1991) Studies on the relative wood decaying capabilities of monokaryotic and dikaryotic mycelia of common decay inducing organisms of Sal (*Shorea robusta)*. Thesis submitted for the Degree of Doctor of Philosophy (Science) of the University of Calcutta.

Norman, A.G. and Jenkins, S.H.(1933) A new method for the determination of cellulose, based upon observation of the removal of Lignin and other Encrusting Materials. Biochem. J., **27**: 818-831.

Wise, Murphy and D'Addico (1946) Cited by Campbell, W.G. in wood chemistry (Cited by Wise, L.E. and John E.C.) 2nd Ed. Vol. **2**. 1952. New York, Rheinhold.

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POSITIVE REGULATION OF HEALTH STATUS AND YIELD ATTRIBUTES OF *Mentha Spicata* USING SALICYLIC ACID

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ABSTRACT

Salicylic acid (SA) is an endogenous signaling molecule that renders plant resistance to biotic and abiotic stresses. It is also involved in potential modulation of growth and yield parameters of crop plants. In this experimental work, an attempt was made to improve the general vigour status, some growth, biochemical variables and foliar yield attributes of a high value medicinal and aromatic plant mint (*Mentha spicata*) by using SA. As the leaves are the main storehouse of secondary metabolites like menthol, carvone, cineol, limonene, dihydrocarvone etc. we tried to enhance mainly the foliar productivity of *Mentha spicata.* After a screening experiment using different concentrations of SA, 100 and 200 µg/ml of this chemical were selected and applied by foliar treatment on *Mentha* plants for two consecutive days after 30 days of planting (DAP). Some growth parameters, measured in terms of shoot and internodal length, leaf and branch number per plant, leaf biomass and leaf area, were recorded after 30 days of foliar treatment. Biochemical parameters like RNA and chlorophyll contents as well as some enzyme activities *viz.*, catalase, amylase and peroxidase were determined from fully mature leaves of plants after 30 days of foliar treatment. SA at higher concentration (200µg/ml) significantly increased the shoot and internodal length, leaf area, leaf number, leaf biomass and branch number per plant when compared with control plants. The macromolecule levels of RNA and chlorophyll as well as enzyme activities like catalase, amylase and peroxidase were increased in the experimental SA treated plants.

INTRODUCTION

In recent years, in both developed and developing countries, the demand of essential oil bearing medicinal plants has increased rapidly. The supply of essential oil is severely lagging behind its demand and it is the need of the day to maximize the essential oil yield of medicinal plants (Naeem *et al.*, 2011). Out of a large number of essential oil bearing plants, mint is an important genus having 25-30 species. India is the largest mint oil producer, with an annual production of essential oil of 15,000-20,000 tons (Chand *et al.* 2004).

M. spicata is one of the potent essential oil yielding species of the genus *Mentha* growing in West Bengal. The extracts of mint species are used in cosmetic industry, food industry, pharmaceutical industry and are generally considered safe to use (Salman *et al.*, 2015) The essential oil of *M. spicata* presents a characteristic spearmint odour (Jirovetz *et al.,* 2002). Carvone is the main volatile component of *M. spicata* (Chauhan *et al.*, 2009).

SA is a non-conventional plant growth regulator (PGR) commonly known as an endogenous signaling molecule and also a phenolic inhibitor which is involved in various physiological processes in plants. It renders plant resistance and tolerance to biotic and abiotic stresses (Popova *et al.*, 1997; Hayat *et al.*, 2010). Several studies also revealed the growth promoting effect of SA (Gharib, 2006). Moreover, exogenous application of SA has been effective in inducing secondary metabolite formation (Kiddle *et al.*, 1994).

In view of the ever-increasing demand of essential oil of mint and the effect of SA in enhancement of growth attributes, the present study was aimed to examine whether the foliar application of two different concentrations of SA could enhance the health status and foliar yield attributes of mint, which might lead to

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oil productivity. Thus, the prime objective of this work was to analyse the role of SA on augmentation of the plant biomass with concomitant enhancement of oil.

MATERIALS AND METHODS

The experiment was conducted in experimental field of Botany Department, Burdwan University and the plant material (*Mentha spicata)* was procured from the Medicinal plant Garden of North Bengal University. Uniformly sized explants having a few nodes in each planting material were planted in the experimental field having loamy soil enriched with vermicompost. The main plot was divided into subplots of one square meter each and interrow as well as interplant spacing in each row was maintained 25x25 cm (i.e. plant-to-plant and row-to-row). And the experiment was done in Randomized Block Design (RBD) with respect to treatments and replications. Different concentrations (0, 100, 200 µg/ml) of SA were applied on the plants through foliar spraying after 30 days of planting. Data were recorded at 30 days after treatment. Fresh weight of *Mentha* leaves was recorded by weighing all plant leaves of 10 uniformly growing plants using an electronic balance. After taking fresh weight of such leaves from each treatment, the leaves were dried at 60ÚC for 48h using a hot air oven and the dry weight of leaves was recorded thereafter. Leaf area was determined by measuring the maximum length and breadth of all the leaves of a plant and multiplying them with a predetermined factor of 0.72 which was critically determined by using graph paper. In fact, the factor was calculated by measuring the actual area of all the leaves of a plant separately on graph paper followed by dividing the respective leaf area with its multiplied product of length and breadth. This process was sufficiently replicated and the average value was used (Bhattacharjee, 1984). Some selected biochemical parameters were taken from mature leaves. RNA contents were measured following the method of Cherry (1962) modified by Choudhuri and Chatterjee (1970). The chlorophyll content was estimated following Arnon's (1949) principle. Catalase activity was determined following the method Snell and Snell (1971). Amylase activity was estimated as per the method of Khan and Faust (1967). Peroxidase activity was assayed following the method of Kar and Mishra (1976). Each experiment was done in three replicates. Statistical analysis in terms of least significant difference (LSD, p=0.05) was done following the method of Panse and Sukhatme (1967).

Table 1.

Effect of different concentrations (0, 100 and 200µg/ml) of salicylic acid (SA) on shoot length (cm), internodal length (cm) and leaf biomass (g) of *Mentha* plants which underwent foliar treatment for two consecutive days from 30 days of plant age. Data were recorded after 30 days of treatment

Treatments	Concentrations (µg/ml)	Shoot length (cm)	Internodal length (cm)	Leaf biomass per plant	
				Fresh wt. (g)	Dry wt. (g)
CONTROL	0	24.11	1.74	10.66	1.94
SA	100	26.42	2.20	15.66	3.01
	200	34.56	2.58	16.50	3.54
LSD		1.80	0.15	0.95	0.14

Table 2.

Effect of different concentrations (0, 100 and 200 µg/ml) of salicylic acid (SA) on leaf number and branch number per plant as well as total leaf area (cm²) of *Mentha* which underwent foliar treatment for two consecutive days from 30 days of plant age. Data were recorded after 30 days of treatment.

Treatments	Concentrations (µg/ml)	Leaf number per plant	Branch number per plant	Total Leaf area (cm^2)
CONTROL	0	49.52	6.78	162.426
SA	100	125.92	16.46	591.824
	200	156.06	18.54	756.891
LSD		10.56	0.74	16.55

RESULTS AND DISCUSSION

Results clearly revealed that SA treatment at both concentrations (100 and 200 µg/ml) increased the shoot and internodal length as well as leaf dry weight when compared with control plants (Table 1). In case of leaf number and branch number per plant as well as total leaf area (cm²) SA treatment increased the growth parameters over the control plant sample, and 200 µg/ml concentration showed best result for enhancement of the variables analysed (Table 2). The biochemical parameters like RNA and chlorophyll levels as well as enzyme (catalase, peroxidase and amylase) activities were increased in the selected PGR treated plants gradually with concentrations (Fig. 1 and 2) against control.

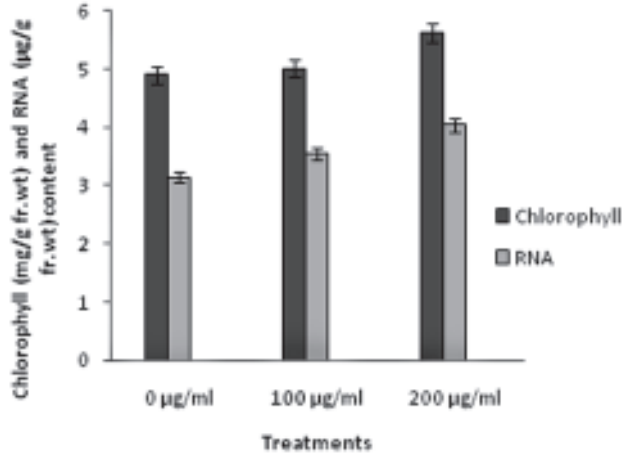

Fig. 1. Effect of different concentrations (0, 100 and 200µg/ml) of salicylic acid (SA) on total RNA and total chlorophyll contents of *Mentha* which underwent foliar treatment for two consecutive days from 30 days of plant age. Data were recorded after 30 days of treatment

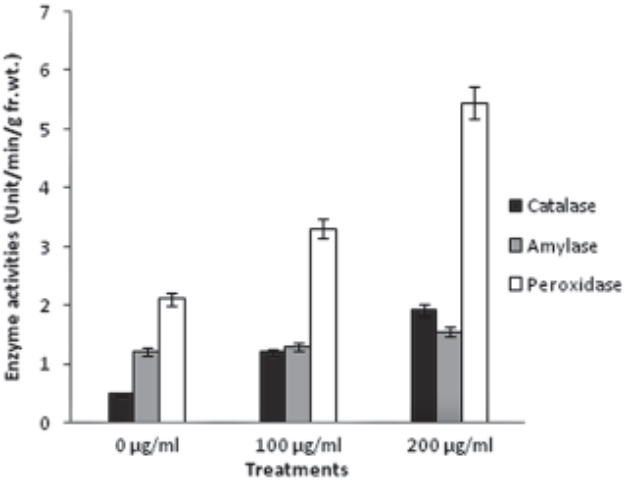

Fig. 2. Effect of different concentrations (0, 100 and 200µg/ml) of SA on enzyme (catalase, amylase, and peroxidase) activities (unit/min/g fr.wt.) of *Mentha* which underwent foliar treatment for two consecutive days from 30 days of plant age. Data were recorded after 30 days of treatment

The use of plant growth regulators on mint plant to improve vegetative growth, metabolism and essential oil productivity were documented earlier (Zheljazkov *et al.,* 2010; Naeem *et al.*, 2011, 2012; Hassanpour *et al.*, 2012; Saharkhiz and Goudarzi, 2014). Application of an aqueous solution of selected plant growth regulator (PGR) SA on shoots of soybean significantly promoted the growth of the plant (Eraslan *et al.*, 2007). It was reported that exogenous application of SA can also increase the essential oil content of mint plant by stimulating their vegetative growth, dry weight and increase of photosynthetic pigments (Abdou and Mohamed, 2014) In the current study, increased growth attributes like shoot and internodal length, total leaf area, leaf and branch number per plant and fresh and dry weight of leaves with concomitant increase of chlorophyll and RNA contents as well as activities of catalase, amylase and peroxidase in SA treated plant samples are well established from repeated experiment. And promotive influence of some phenolic compounds including SA at specific concentrations is available from some previous reports (Gharib, 2006; Kim *et al.*, 2009). In this experiment, increased total leaf area, leaf and branch number per plant as well as fresh and dry weight leaves in SA treated plants indicated the overall augmentation of foliar productivity of the test plant. Thus, by virtue of increasing the biomass of leaves by SA treatment along with its enhancement of metabolite potential, as evidenced from some reliable biochemical parameters, it can be concluded that SA is potential enough for exhibiting better field performance which consequently resulted in coveted productivity of the experimental *Mentha* species.

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REFERENCES

Abdou M. and Mohamed M. A. H. (2014). Effect of compost, salicylic and ascorbic acid on *Mentha piperita* L. plants. *Biological Agriculture & Horticulture*. **30**(2): 131-143.

Arnon D. J. (1949). Copper enzymes in isolated chloroplasts, Polyphenol-oxidase in *Beta vulgaris*. *Plant Physiol*. **24**: 1-15.

Bhattacharjee A. (1984). *Responses of sunflower plants towards growth retardants with special reference to growth, metabolism and yield*. Ph.D. Thesis, Burdwan University, West Bengal, India.

Chand S., Patra N. K., Anwar M., Patra D. D. (2004). Agronomy and uses of menthol mint (*Mentha arvensis*)-Indian perspective. *Proc Indian Natural Sci Acad*. B**70**(3): 269-297.

Chauhan R. S., Kaula M. K., Shahia A. K., Kumara A., Rama G. and Tawab A. (2009). Chemical composition of essential oils in *Mentha spicata* L. accession (IIIM[J]26) from North-West Himalayan region. *Indian Ind. Crops Prod*. **29**:654-656.

Cherry J. H. (1962). Nucleic acid determination in storage tissue of higher plants. *Plant Physiol.* **37**: 670-678.

Choudhuri M. A. and Chatterjee S. K. (1970). Seasonal changes and the levels of some cellular components in the abscission zone of *Coleus* leaves of different ages. *Ann. Bot.* **34**: 275-287.

Earsalan F., Inal A., Gunes A. and Alpaslan M. (2007). Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Scientia Hort*. **113**: 120-128.

Gharib F. A. L. (2006). Effect of salicylic acid on growth, metabolic activities and oil content of Basil and Marjoram. *Int J of Agri and Biol*. **8**(4): 485-492.

Hassanpour H., Nejad R. A. K., Niknam V., Najafi F. and Razavi K. (2012). Effects of penconazol and water deficit stress on physiological and antioxidative responses in pennyroyal (*Mentha pulegium* L.). *Acta Physiol Plant*. **34**: 1537-1549.

Hayat Q., Hayat S., Irfan M. and Ahmad A. (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environ. Exp. Bot*. **68**: 14-25.

Jitrovetz L., Buchbauer G., Shabi M. and Ngassoum M. B. (2002). Comparative investigation of essential oil and volatiles of spearmint. *Perfum. Flav*. **27**: 16-22.

Kar M. and Mishra D. (1976) Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence. *Plant physiology*. **57**: 315-600.

Khan A. A. and Faust M. A. (1967). Effect of growth retardants on amylase production in germination barely seeds. *Physiologica Plantarum*. **20**: 673-681.

Kiddle G. A., Doughty K. J. and Wallsgrove R. M. (1994). Salicylic acid-induced accumulation of glucosinolates in oilseed rape (*Brassica napus* L.) leaves. *J Exp Bot*. **45**: 1343-1346.

Kim H. Y., Hamayun M., Khan A. L., Na C. I., Kang S. M., Han H. H. and Lee I. J. (2009). Exogenous application of plant growth regulators increased the total flavonoid content in *Taxacum officinale* Wigg. *African J of Biotech.* **8**(21): 5727-5732.

Naeem M., Idrees Mohd., Alam M. M., Aftab T. Masroor M., Khan A. and Moinuddin. (2012). Brassinosteroid-mediated enrichment in yield attributes active constituents and essential oil production in *Mentha arvensis* L. *Russian Agri. Sci.* **38**(2): 106-113.

Naeem M., Masroor M., Khan A., Moinuddin, Idrees Mohd. and Aftab T.. (2011). Triacontanol- mediated regulation of growth and other physiological attributes, active constituents and yield of *Mentha arvensis* L. *Plant growth Regul*. **65**: 195-206.

Panse, V. G. and Sukhatme, P. T. (1967). Stastical method for agricultural works. ICRA, New Delhi, India. 150-157.

Popova L., Pancheva T. and Uzunova A. (1997). Salicylic acid: properties, biosynthesis and physiological role. *Bulg. J. Plant Physiol*. **23**: 85-93.

Saharkiz M. J. and Goudarzi T. (2014). Foliar application of salicylic acid changes essential oil content and chemical compositions of peppermint (*Mentha piperita* L.). *Journal of essential oil bearing plants.* **17**(3): 435-440.

Salman M., Abdel-Hameed El-S. S., Bazaid S. A. and Dabi M. M. (2015). Chemical composition for hydrodistillation essential oil of *Mentha longifolia* by gas chromatography-mass spectrometry from north regions in Kingdom of Saudi Arabia. *Der Pharma Chemica*. **7**(4): 34-40.

Snell F. D. and Snell C. T. (1971). Colorimetric methods of analysis. Van Nostrand Reinhold Co., New York, IV AAA, 7-145.

Zhelijazkov V. D., Astatkie T., Horgan T. and Rogers S. M. (2010). Effect of plant hormone and distillation water on mints. *Hort Science*. **45**(9): 1338-1340.

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EXOPOLYSACCHARIDE PRODUCTION BY A RHIZOBIUM SP. FROM ROOT NODULES OF *Phaseolus mungo* (L.) IN HEAVY METAL STRESS CONDITION

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ABSTRACT

Rhizobia are Gram negative bacteria that can exist either as free-living bacteria or as nitrogen fixing symbionts inside the root nodules of leguminous plants. Among rhizobial polymers, exopolysaccharides (EPS) play a key role and is indispensable for the invasion of a great majority of host leguminous plants which form root nodule. In the present investigation, initially ten bacterial strains were isolated from the root nodule of *Phaseolus mungo* (L.), and all were identified as a species of *Rhizobium* through different physiological and biochemical tests. All the strains produced EPS in yeast extract Mannitol (YEM) medium. But strain P3 produced maximum amount (470.0µg/ml) and reached its stationary phase at 24 hours. The isolate preferred pH 6.9 and sucrose (1.0%) for maximum EPS production. The effect of five heavy metals (viz. Cd, Ba, Pb, Hg and Ag) on growth and EPS production of the isolate was checked. All the tested heavy metals had inhibitory effect at higher concentration (100µg/ml), but the inhibitory effect of some heavy metals at lower concentration can be overcome by this strain. The ability of the isolated bacteria to grow and to produce EPS in heavy metal stress condition might be utilized for the production of local biofertilizer rhizobial strain which might be helpful in sustainable agriculture.

INTRODUCTION

A leguminous plant is important both ecologically and agriculturally, since it is a major source of biological nitrogen fixation through legume-rhizobia symbiosis as well as they are also rich source of vegetative protein, especially by the pulse cropses. Rhizobia comprise a very diverse group of nitrogen-fixing symbiotic bacteria that belong to á and â subclasses of the Proteobacteria and are members of several genera, including *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium*, *Allorhizobium* and *Methylobacterium* (á-rhizobia), as well as the members of â-rhizobia like *Burkholderia* and *Cupriavidus* (Dresler-Nurmi *et al.* 2009). Different aspects of legume-rhizobia symbiosis were studied time to time, but more emphasis was given to nitrogen fixation. But the production of exopolysaccharide (EPS) by the rhizobia is also an important aspect in legume-rhizobia symbiosis. Rhizobial polysaccharides are necessary for establishing symbiotic associations (Hoang *et al.* 2004). Besides, rhizobial EPS may also be involved in invasion and nodule development, bacterial release from infection threads, bacteroid development, suppression of plant defense response and plant antimicrobial compounds (Skorupska *et al.* 2006). The microbial polysaccharide has also generated increasing attention among researchers for the last couple of years because of its commercial interest, especially in hydrocarbondegradation (Huang *et al.* 2012; Han *et al.* 2014) and biomedical field (Roberts 1995). In the recent years, the toxicity of heavy metals and organic pollutants to rhizobia and soil bacteria is a field of intense research. This is due to application of industrial waste water and sewage to agricultural land (Wei *et al.* 1985). But most of the work has been done on the effect of heavy metals on the soil population of rhizobia (Chaudri *et al.* 2008), nodulation (Paudyal *et al.* 2007), nitrogen fixation (Ibekwe *et al.* 1996) and also on rhizobial indole acetic acid (IAA) production (Bhattacharyya 2006). But the report on heavy metals on rhizobial exopolysaccharide production which play an important role in legume-rhizobia symbiosis, has not been studied earlier. Present investigation aims to screen the maximum EPS producing strain of *Rhizobium* from *P. mungo* and to check the effect of heavy metals on growth and EPS production by the *Rhizobium* sp.

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MATERIALS AND METHODS

Plant material

A pulse crop, *Phaseolus mungo* L. was selected for the present study.

Isolation of microsymbiont, medium and growth condition

For the isolation of microsymbiont, pink-coloured root nodules of *P. mungo* were selected. Properly washed and surface sterilized root nodules were crushed and streaked on Yeast Extract Mannitol (YEM) agar medium of Skerman (1956) with slight modification, and incubated at 30 $\pm$ 2° C for 2 days. The isolated colonies (strains) were identified as a fast growing species of *Rhizobium* following Jordan (1984). For growth study, bacteria were incubated in 20ml of YEM broth at 302^0 C for 24 hours (optimum time for EPS production). Growth was measured spectrophotometrically at 540nm.

Isolation and Purification of EPS

For the extraction of EPS from the bacterial culture, the solvent extraction method of Djordjevic *et al.* (1986) was followed with some modifications. Samples of cultures broth were centrifuged (12,000g, 30min), supernatants were collected and was mixed with thrice volume of cold ethanol and stood overnight prior to centrifugation. Then the resulting precipitate was collected by centrifugation at 10,000g for 30min, re-dissolved in distilled water, and dialysed through a cellulose membrane (Sigma-Aldrich, retaining MW> 12,400 Da) against deionized distilled water for 24 hr to remove low molecular weight materials. The dialyzed material was lyophilized to obtain purified EPS.

Estimation of EPS

For estimation of EPS, phenol-sulfuric acid method of Dubois *et al.* (1956) was followed. One ml of purified EPS was mixed with 1ml of 5% (v/v) phenol and then 95% (v/v) concentrated sulfuric acid was added, shaken well and kept at room temperature for 25 minutes. The brown colour developed was measured at 490nm in a spectrophotometer. The concentration of EPS was calculated from a standard curve prepared by glucose.

Optimization of carbon source, pH and the effect of different heavy metals on EPS production

For optimization of carbon source for maximum EPS production, the basal yeast extract mineral medium was supplemented with filter-sterilized different carbon sources at 1% (v/v) level. Then individual effect of carbon sources on EPS production was checked. For optimum pH requirements of EPS production, the medium was adjusted with different pH and EPS was measured as described previously. To check the effect of different heavy metals on EPS production, the concentration of tested heavy metals were added to yeast extract mineral medium with optimized carbon source and pH. The EPS was measured as the method written above.

Statistical analysis

Statistical analysis were done according to Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

From the root nodules of *P. mungo* , symbionts were isolated in YEM agar plate. Initially, ten strains were selected and all are species of *Rhizobium.* All the strains were tested for their EPS production and it was found that all strains had the capacity to EPS production in YEM broth, but in different extent (Table 1). The strain no. P3 was found the best producer of EPS. For this, successive experiments were carried out with the P3 strain. This P3 strain of *Rhizobium* is fast growing species which reached its stationary phase for both growth and EPS production at 24 h (Fig.1). The effect of different carbon sources on growth and EPS production was also tested. From the Table 2 it was found that sucrose was the best carbon source utilized for EPS production. The optimum concentration of preferred carbon source (sucrose) was 1.0% (Fig.2). The optimum pH requirement for both growth and EPS production was found 6.9 (Fig.3). The effect of different heavy metals on the growth and EPS production by the P3 rhizobia was checked (Table 3). From the result it was

Table 1.

EPS production by the different strains of *Rhizobium* from the root nodules of *P. mungo.*

Strains of *Rhizobium*	EPS Production (µg/ml)
P1	400.0
P2	360.0
P3	470.0
P4	315.0
P5	430.0
P6	420.0
P7	390.0
P8	410.0
P9	400.0
P10	415.0
CD, P=0.05	

The bacteria were grown in YEM medium (pH 7.0) for 24 h at 302°C. Data presented here are the mean of three experimental sets

Conditions were as in Table 1

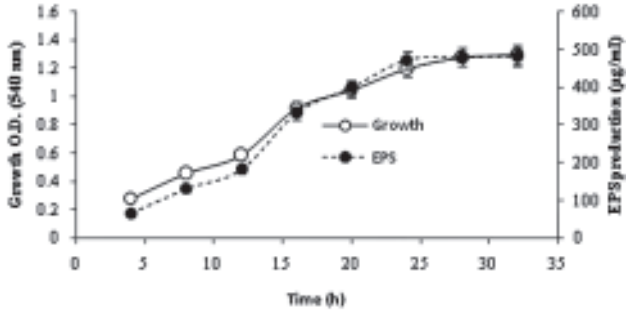

Fig. 1. Changes in growth with EPS production on YEM medium at pH 7.0 by P3 *Rhizobium*

found that all the tested heavy metals had the inhibitory effect on both growth and EPS production at higher concentration. Most inhibitory effect on EPS production was found with Cd (56.7%) and Hg (56.5%) over control. But however, there were promotive effect on EPS production by this P3 strain with Ba and Pb, at least in lower concentration.

Microsymbiont was isolated from the healthy and pink coloured root nodules of *P. mungo*. Initially ten strains (designated as P1,P2,…P10) were isolated, and all were identified as a fast growing species of *Rhizobium* according to Jordan (1984). All these ten strains were checked for congo red absorbtion test (Eissa *et al.* 2009) to ensure that all isolates were *Rhizobium* and not mixed with *Agrobacterium*. The characteristic features of growth, colony morphology and as well as cell morphology of the isolated strains were examined to confirm their purity and probable differences among themselves.

All the ten isolated rhizobial strains were checked for their EPS production ability in YEM broth. From the Table 1 it was found all the strains had the capacity to produce EPS, though in different extent. The highest (470µg/ml) production was obtained in P3 strain. For this, the successive experiments were carried out with P3 strain.

The *Rhizobium* P3 reached its stationary phase for growth within 24 h at an optimum inoculum dose (Fig.1). So, it can be said that isolated strain is a fast growing species. The *Rhizobium* P3 also produced maximum EPS within 24 h (Fig.1). Maximum EPS production at the onset of stationary phase was also reported earlier (De and Basu, 1996). Growth and EPS production started simultaneously from the very beginning (Fig. 1) indicated that a part of the EPS might act as primary metabolite and help in nodulation.

It was reported earlier (Yuksekdag and Aslim, 2008; Nirmala *et al.*, 2011) that the characteristics of EPS and its amount can be mostly influenced by the nature of carbone source and its concentration supplemented within the media. For this, we have checked the effect of different carbon sources on growth and EPS production by the *Rhizobium* P3. From the table 2 it was found that all the tested carbon sources can be utilized for both growth and EPS production. However, maximum growth was obtained with glucose. On the other hand, the yield of EPS was maximum with glucose and also with sucrose. But if specific productivity (EPS production/growth) is considered, then sucrose is the best carbon source for the EPS production (Table 2).

Table 2

Effect of different carbon sources on growth and EPS production by the *Rhizobium* (P3)

Carbone sources	Growth O.D. at 540 nm	EPS production (µg/ml)	Specific Productivity (EPS production/growth)
Control	0.53	160.8	303.4
D-Ribose	1.00	356.5	356.5
D-Galactose	1.05	395.0	376.2
L-Arabinose	1.10	377.0	342.8
D-Xylose	1.10	418.0	380.0
Mannitol	1.20	475.5	396.3
Sucrose	1.15	480.5	417.8
D-Glucose	1.30	480.0	369.2
D-Fructose	1.20	452.8	377.3
Myoinositol	0.95	363.5	382.6
Maltose	1.00	406.5	406.5
CD at P=0.05	0.04	4.14	2.45

Control set devoid of carbon sources. In other sets, carbon sources (1.0% level) were added individually in yeast extract mineral medium. Other conditions were as Table 1.

Sutherland (2001), Castellane and Lemos (2007) also reported that for the production of microbial EPS, sugars like glucose or sucrose are usually used as carbon sources because they allow for high productivities and yield. However, Castellane *et al.* (2015) are of the opinion that glucose seems not to be a good carbon source for EPS production, since in some bacteria including rhizobia, this compound is engaged in catebolite repression. In addition, our data are in accord with the report of Castellane *et al.* (2014) who reported that *R. tropici* and rhizobial isolates from *P. vulgaris* L. grows and produces measurable EPS using sucrose as a carbon source. In respect of concentration, it was found that 1% sucrose was optimum for maximum EPS production (Fig.2). Datta and Basu (1999) also reported that for optimum EPS production by a *Rhizobium*, 1% glucose is needed.

Conditions were as in Table 2

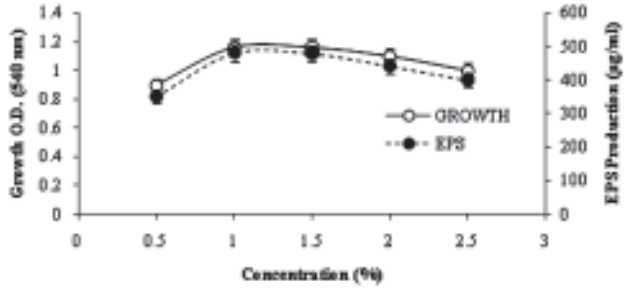

Fig. 2. Effect of different concentration of most preferred carbon source (sucrose) on growth and EPS production by the P3 *Rhizobium.*

The bacteria were grown in yeast extract mineral medium supplemented with 1% sucrose. Other conditions were same as in Table 2.

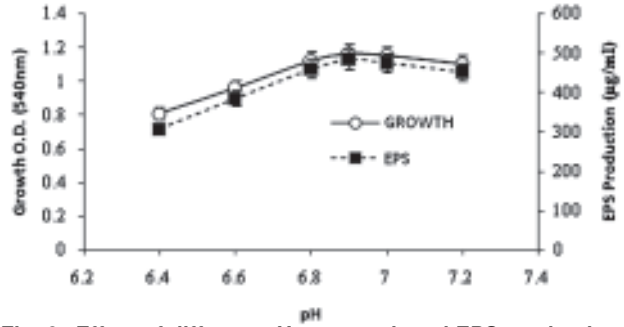

Fig. 3. Effect of different pH on growth and EPS production by the P3 *Rhizobium*

For optimum growth and EPS production by the *Rhizobium* P3, the pH requirement was checked. From the Fig.3, it was found that isolate mostly preferred pH 6.9 for both growth and EPS production. However, it is also clear that the isolate can grow and produce EPS with a wide range of pH (pH 6.4 to 7.2). These findings agreed with the reported range of pH (5.0 to 8.5) tolerance for the growth of *Rhizobium* (Bowra and Dilworth, 1981) and also other secondary metabolite (IAA) production by *Rhizobium* (Bhattacharyya *et al.* 2004). Martensson (1992) reported that most of the cultivated legumes are exposed to agrichemicals and fertilizers which not only contain essential nutrients but also contaminants such as heavy metals. Keeping this comment in mind, we have checked the effect of different heavy metals on growth and EPS production by the *Rhizobium* P3. From the Table 3 it was found that out of five heavy metals tested, the Cd and Hg had the negative effect on both growth and EPS production, irrespective of concentration used. But, other three (viz. Ba, Pb and Ag) heavy metals had some promotive effect on EPS production, at least in lower concentration. However, the maximum positive effect was obtained in EPS production (13.7% over control) and growth (12.5%) by Pb at the concentration of 5µg/ml. Abd-Alla *et al.* (1999) reported that at high application rates(40 and 50%), sewage sludge significantly inhibited nodulation, nitrogen fixation and nitrogen yields of fababean and soybean, which was most probably due to toxic effect of heavy metals (Cu and Zn) on the microsymbiont rather than host plant. Musarrat and Haseeb (2000) are of the opinion that agrichemicals may protect *Rhizobium* recognition sites on the root surface of legumes. As a result, the biological nitrogen fixation and consequently the yield of leguminous crop will be decreased due to reduced nodulation.

Exopolysaccharides produced by rhizobia are especially important for the successful development of legume-rhizobia symbiosis. Skorupska *et al.* (2006) reported that EPS produced by rhizobia played an essential and significant role in proper functioning of legume-rhizobia symbiosis. It was reported that mutants of *R. meliloti* that fail to produce EPS (Exo^-) induce empty, non-nitrogen fixing (Fix^-) nodules on alfalfa roots (Leigh *et al.* 1985).

Apart from the role in the development of successful legume-rhizobiosis, the EPS has also several important role in the field of environment. A good number of workers reported that microbial EPS can be used as bioemulsifiers and increase the solubilities of hydrocarbon degradation (Huang *et al.* 2012), bioflocculating (Mandal *et al.* 2013) and bioadsorption of heavy metals from wastewater and natural water (Shuhong *et al.* 2014). EPS secreted by microorganisms are recommended as surface active agents for bioremediation of heavy metals (Pagnanelli *et al.* 2000). Again EPS rich broth promoted seed germination, shoot length, root length, number of leaves and chlorophyll content of wheat and peanut seeds (Sayyed *et al.* 2015).

Table 3

Effect of heavy metals on growth and EPS by the P3 isolate.

Heavy Metals	Concentration (µg/ml)	Growth O.D. (540 nm)	% increase or % decrease over the control	EPS production (µg/ml)	% increase or % decrease over the control
Control	–	1.2	–	480.0	–
Cd ($CdCl_2$)	5	0.97	(-)9.2	390	(-)18.8
	25	0.78	(-)35.0	300	(-)37.5
	50	0.63	(-)47.5	250	(-)47.9
	100	0.54	(-)55.0	208	(-)56.7
Ba($BaCl_{2)}$	5	1.25	(+)4.2	498.3	(+)3.8
	25	1.23	(+)2.5	492.5	(+)2.6
	50	1.20	(+)0.0	481.7	(+)0.4
	100	1.17	(-)2.5	471.4	(-)1.8
Pb($PbCl_2$)	5	1.35	(+)12.5	545.8	(+)13.7
	25	1.33	(+)10.8	532.1	(+)10.9
	50	1.25	(+)6.0	500.8	(+)4.3
	100	1.18	(-)1.6	478.6	(-)0.3
Hg(Hg Cl_2)	5	0.72	(-)40.0	290.0	(-)39.6
	25	0.68	(-)43.3	275.5	(-)42.6
	50	0.64	(-)46.7	250.3	(-)47.9
	100	0.51	(-)57.5	208.8	(-)56.5
Ag ($AgNO_3$)	5	1.12	(-)6.7	482.6	(+)0.54
	25	1.22	(+)1.7	493.0	(+)2.71
	50	1.11	(-)0.75	477.7	(-)0.48
	100	0.92	(-)23.3	400.8	(-)16.5

The bacteria were grown at pH 6.9 in yeast extract mineral medium supplemented with 1.0% sucrose and heavy metals were added individually in the medium. Control sets contained without any heavy metals. Other conditions were same as in Table 2.

So, from the above results and discussion it can be said that:

a) The isolated *Rhizobium* P3 have the capacity to produce high amount of EPS which can be used as seed inoculants for the better crop yield.

b) The growth and EPS production by the isolated rhizobia in the presence of some of the heavy metals tested indicated the heavy metals resistance of the isolate. This isolate can be used for producing heavy metal resistant biofertilizer strain, particularly heavy metal contaminated soil.

c) The EPS produced by this isolated rhizobia might be utilized for some remediation of environmental problems.

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REFERENCES

Abd-Alla, M. H., Yan, F. and Schubert, S. (1999). Effects of Sewage Sludge Application on Nodulation, Nitrogen Fixation and Plant Growth of Faba Bean, Soybean, and Lupin. Journal of Applied Botany, **73**, 69-75.

Bhattacharyya, R. N. (2006). Effects of heavy metals on growth and indole acetic acid production by Rhizobium sp. Bangladesh J. Bot. **35(1)**, 63-69.

Bhattacharyya, R.N., Dan, U.K. and Chandra, K. (2004). Role of heavy metals on growth and indole acetic acid production by a *Rhizobium* sp. isolated from the root nodules of a leguminous tree from Darjeeling hills. Journal of Science and Techn. Sambalpur University **16(A)**: 86-92.

Bowra, B. J. and Dilworth, M. J. (1981). Motility and chemotaxis towards sugars in *Rhizobium leguminosarum*. Journal of General Microbiology, **126**(1), 231-235.

Castellane, T. C. L. and de Macedo Lemos, E. G. (2007). Notas Científicas Composição de exopolissacarídeos

produzidos por estirpes de rizóbios cultivados em diferentes fontes de carbono. Pesq. Agropec. Bras., Brasília, **42**(10), 1503-1506.

Castellane, T. C. L., Lemos, M. V. F. and de Macedo Lemos, E. G. (2014). Evaluation of the biotechnological potential of Rhizobium tropici strains for exopolysaccharide production. Carbohydrate polymers, **111**, 191-197.

Castellane, T. C. L., Persona, M. R., Campanharo, J. C. and de Macedo Lemos, E. G. (2015). Production of exopolysaccharide from rhizobia with potential biotechnological and bioremediation applications. International journal of biological macromolecules, **74**, 515-522.

Chaudri, A. M., Allain, C. M., Barbosa-Jefferson, V. L., Nicholson, F. A., Chambers, B. J. and McGrath, S. P. (2000). A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-term field experiment. Plant and Soil, **221**(2), 167-179.

Datta, C. and Basu, P. S. (1999). Production of extracellular polysaccharides by a Rhizobium species from the root nodules of *Melilotus alba*. Acta Biotechnologica, **19**(4), 331-339.

De, P. S. and Basu, P. S. (1996). Production of extracellular polysaccharides by a Rhizobium species from the root nodules of *Tephrosia purpurea* Pers. Acta Biotechnologica, **16**(2 3), 155-162.

Djordjevic, S. P., Rolfe, B. G., Batley, M. and Redmond, J. W. (1986). The structure of the exopolysaccharide from Rhizobium sp. strain ANU280 (NGR234). Carbohydrate research, **148**(1), 87-99.

Dresler-Nurmi, A., Fewer, D. P., Räsänen, L. A., and Lindström, K. (2009). The diversity and evolution of rhizobia. In *Prokaryotic Symbionts in Plants* (pp. 3-41). Springer Berlin Heidelberg.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances.Analytical Chemistry, **28**(3), 350-356.

Eissa, A. R., Abd-El-Bary, M. E., Fahmi, A. I., Nagaty, H. H. and Hassan, M. M. (2009). Evaluation and characterization of ã induced mutants of *Rhizobium leguminosarum* in *Vicia faba*. In *Proceedings of the 1st Nile Delta Conference,(NDC'09), Minufiya University, Egypt* (pp. 157-170).

Han, P. P., Sun, Y., Wu, X. Y., Yuan, Y. J., Dai, Y. J. and Jia, S. R. (2014). Emulsifying, flocculating, and physicochemical properties of exopolysaccharide produced by cyanobacterium *Nostoc flagelliforme*. Applied Biochemistry and Biotechnology, **172**(1), 36-49.

Hoang, H. H., Becker, A. and González, J. E. (2004). The LuxR homolog ExpR, in combination with the Sin quorum sensing system, plays a central role in Sinorhizobium meliloti gene expression. Journal of Bacteriology, **186**(16), 5460-5472.

Huang, K. H., Chen, B. Y., Shen, F. T. and Young, C. C. (2012). Optimization of exopolysaccharide production and diesel oil emulsifying properties in root nodulating bacteria. World Journal of Microbiology and Biotechnology, **28**(4), 1367-1373.

Ibekwe, A. M., Angle, J. S., Chaney, R. L. and Van Berkum, P. (1996). Zinc and cadmium toxicity to alfalfa and its microsymbiont. Journal of environmental quality, **25**(5), 1032-1040.

Jordan, D. C. (1984). Family III. Rhizobiaceae. Bergey's manual of systematic bacteriology, **1**, 234-242.

Leigh, J. A., Signer, E. R. and Walker, G. C. (1985). Exopolysaccharide-deficient mutants of Rhizobium meliloti that form ineffective nodules. Proceedings of the National Academy of Sciences, **82**(18), 6231-6235.

Mandal, A. K., Yadav, K. K., Sen, I. K., Kumar, A., Chakraborti, S., Islam, S. S. and Chakraborty, R. (2013). Partial characterization and flocculating behavior of an exopolysaccharide produced in nutrient-poor medium by a facultative oligotroph *Klebsiella* sp. PB12. *Journal of bioscience and bioengineering*, **115**(1), 76-81.

Mårtensson, A. M. (1992). Effects of agrichemicals and heavy metals on fast-growing rhizobia and their symbiosis with small-seeded legumes. *Soil Biology and Biochemistry*, **24**(5), 435-445.

Nirmala, P. (2011). Production of Extracellular Polysaccharides by a Rhizobium Species from Root Nodules of *Vigna mungo* (Hepper). International Journal of Pharmaceutical & Biological Archive, **2**(4), 1209-1214.

Pagnanelli, F., Petrangeli Papini, M., Toro, L., Trifoni, M. and Veglio, F. (2000). Biosorption of metal ions on Arthrobacter sp.: biomass characterization and biosorption modeling. Environmental Science & Technology, **34**(13), 2773-2778.

Panse, V.G. and Sukhatme, P.V. (1985). Statistical methods for agricultural workers (4th Eds.), Indian Council of Agricultural Research, N.Delhi,India.

Paudyal, S. P., Aryal, R. R., Chauhan, S. V. S. and Maheshwari, D. K. (2007). Effect of heavy metals on growth of Rhizobium strains and symbiotic efficiency of two species of tropical legumes. Scientific World, **5**(5), 27-32.

Roberts,I.S. (1995). Bacterial polysaccharides in sickness of in health. Microbiology. **141**: 2023-2031.

Sayyed, R. Z., Patel, P. R. and Shaikh, S. S. (2015). Plant growth promotion and root colonization by EPS producing Enterobacter sp. RZS5 under heavy metal contaminated soil. Indian Journal of Experimental Biology, **53**, 116-123

Shuhong, Y., Meiping, Z., Hong, Y., Han, W., Shan, X., Yan, L. and Jihui, W. (2014). Biosorption of Cu 2+, Pb 2+ and Cr 6+ by a novel exopolysaccharide from Arthrobacter ps-5. Carbohydrate Polymers, **101**, 50-56.

Skerman, V. B. (1959). A guide to the identification of the genera of bacteria. *A guide to the identification of the genera of bacteria.The Williams and Wilkins Co.* Baltimore, 189-191.

Skorupska, A., Janczarek, M., Marczak, M., Mazur, A. and Król, J. (2006). Rhizobial exopolysaccharides: genetic control and symbiotic functions.Microbial Cell Factories, **5**(1), 7-25.

Sutherland, I. W. (2001). Microbial polysaccharides from Gram-negative bacteria. International Dairy Journal, **11**(9), 663-674.

Wei, Q. F., Lowery, B. and Peterson, A. E. (1985). Effect of sludge application on physical properties of a silty clay loam soil. Journal of Environmental Quality, **14**(2), 178-180.

Yuksekdag, Z. N. and Aslim, B. (2008). Influence of Different Carbon Sources on Exopolysaccharide Production by Lactobacillus delbrueckii subsp. bulgaricus (B3, G12) and *Streptococcus thermophilus* (W22). Brazilian Archives of Biology and Technology, **51**(3), 581-585.

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GENETICALLY MODIFIED CROPS

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ABSTRACT

Genetic manipulations of crop plants are an age old practice which began thousands of years ago with the selection of desirable crop plants for cultivation. At the beginning, however, the primitive people relied on chance and thereafter, on experiences to create new variations and selecting new variants with improved characteristics which are inherited in the off spring. Long after that Gregor Mendel discovered the fundamental basis for inheritance of traits in the off springs and laid the foundation stone of modern genetics. Actually, advances made in the application of Mendel's Laws of Genetics resulted in the Green Revolution in 1964. Fundamental discoveries of DNA structure, plasmid, restriction enzymes, plant regeneration, recombinant DNA technology and Ti plasmid collectively advanced the process of genetic transformation of plants with specific targets. Application of DNA based GM technology has opened a new vista for commercial crop production. Such GM crops have generated a lot of interest throughout the world. An attempt has been made in this article to provide an outline of the techniques involved in modifications of the traits of the crop, adoption level of the GM crops their benefits and risks involved.

INTRODUCTION

Genetically modified crops (GMCs, GM crops or biotech crops) are those crops used in commercial crop production, genetic make ups of which have been modified using genetic engineering techniques. GM crops have been modified with various traits which do not occur naturally. For example, in food crops, pest resistance, disease resistance or resistance to environmental aberrations and herbicidal treatment have been achieved in such GM crops. In non- food crops improvement of production of pharmaceutical agents, biofu els and other industrially useful products, including bioremediation, has been achieved through genetic modification.

Genetic manipulations of crop plant is however, not a new phenomenon. It began thousands of years ago when human beings ceased to be nomadic and began to settle, cultivate land and select desirable crop plants. At the beginning, they relied on chance and thereafter, on experience, to create new variation and selecting new variants with improved characteristic which are in herited in the offspring. The credit goes to Gregor Mendel (1822-1884) for the discovery of the fundamental basis for inheritance of traits in the offspring which hold true for all forms of life, including crop plants, and which laid foundation stone of modern genetics. Mendel's laws remained, however, in the dark for 35 years which were rediscovered in 1901. Most of our present day knowledge on Genetics dates back from the time of this rediscovery. The rediscovery of Mendel's laws of genetics opened a new era in crop breeding. Actually, the Green revolution in 1964 was based on the application and subsequent refinement of a single scientific discovery of Mendel's Laws of Genetics. After the fundamental discovery of DNA structure by Watson and Crick (1953) and subsequent discoveries of the plasmid (1959), restriction enzymes(1970), plant regeneration (1970), recombinant DNA technology (Jackson *et al*, 1972;Meynall, 1973) and Ti plasmid collectively made the genetic transmission of plants possible.

Application of recombinant DNA based GM technology has opened up a new vista for raising commercial crop production. However, recombinant DNA based GM technology differs from conventional breeding in that totally new genes are transferred between widely unrelated organisms and the location of these genes at recipient genomes is random, unlike gene transfer taking place in traditional breeding. Such GM crops have generated a lot of interest throughout the world. In this background an attempt has been made in

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this article to have a glimpse of the techniques involved in modification of traits of covered crops, application and adoption level of modified crops including the benefits and risks involved in the adoption of such crops.

GENE TRANSFER TECHNIQUES

There are several natural mechanisms which result gene transfer across species. Antibiotic resistance development in bacteria facilitated by transposons, retrotransposons, proviruses and other mobile genetic elements which naturally translocate DNA to new loci in a genome is the result of one of the natural mechanisms (Lederberg *et al.,* 1946; Bock,2010; Morgante *et al.,* 2005; Long *et al.,* 2003). Such transfer occurs over an evolutionary time scale (Feschotte *et al.,* 2005; Cordaux *et a.l* 2006 and Long *et al.* 2003). Traditional crop breeders achieved transferring foreign germplasm to crops by overcoming species barriers. As early as in 1875 a hybrid cereal grain was developed by crossing wheat and rye (Chen, 2010). Since then important characters, including dwarfing genes, rust resistance have been introduced (Long *et al.*, 2003). Plant tissue culture and deliberate mutations have enabled altering the plant genomes (Predieri, 2001; Duncan, 1996).

Genetical engineering techniques involved in adding or removing genes from the crops include the uses of gene guns, electroporation, microinjection, agrobacterium. More recently CRISPR and TALEN technologies are considered more precise and convenient techniques.

The use of gene guns is the most common method in which the gene guns shoot target genes into plant cells. In this method, DNA is bound to tiny particles of gold or tungsten which are subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and the cell membranes. Thereafter, the DNA separates from the metal and is integrated into plant DNA inside the nucleus. This method has proved to be more successful for many cultivated crops, especially monocots like wheat or maize, where the use of *Agrobacterium tumefaciens*, has been less successful (Shrawat and Lorz, 2006). One disadvantage of this technique, however, is that, it may cause damage to the cellular tissue.

Agrobacterium tumefaciens mediated transformation is another common technique. The natural ability of the plant parasite *Agrobacteria* to transfer gene provides another engineering method. In order to create a suitable environment for themselves, these agrobacteria insert their genes into host plants resulting in a proliferation of modified plant cell near the soil level forming crown gall. The genetic information for tumour growth is encoded on a mobile, circular DNA fragment plasmid. This T-DNA is transferred to a random site in the plant genome when agrobacterium infects a plant. In genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. Acting as a vector, the bacterium transport foreign genes into plants. This method has proved to be successful for dicotyledonous plants like potato, tomato and tobacco. It is less successful in monocotyledonous crops like wheat and maize.

The technique of electroporation is used when the plant tissue does not contain cell walls. In these method, DNA enters the plant cells through pores temporarily caused by electric pulses. Micro injection directly injects the gene into the DNA.

The techniques of CRISPRS and TALEN are much more precise and convenient editing techniques. CRISPRS- Clustered Regularly Interspaced Short Palindromic Repeats, are segments of prokaryotic DNA containing repetitions of the base sequences. TALEN-Transcription Activator Like Effector Nuclease, are artificial restriction enzymes generated byfusing a TAL effector DNA- binding domain to a DNA cleavage domain. By combining such an engineered TALE with a DNA cleavage domain (which cuts DNA strands), one can engineer restriction enzymes which are specific for any desired DNA sequence. When these restriction enzyme are introduced into cells, they can be used for genome editing with engineered nucleases.

A promoter specific to the area where the gene is to be expressed is required with introducing new gene into plants. For example, to express a gene only in rice grain and not in leaves an endosperm specific promoter is used. The codons of the gene must be optimized for the organism due to codon usage bias. Transgenic gene products should be able to be denatured by heat, so that they are destroyed during cooking. Result of modern comprehensive profiling of crop composition suggest that crops modified using GM techniques are less likely to have unintended changes then are conventionally bred crops. (Catchpole *et al* 2005)

Arabidopsis thaliana and tobacco are the most frequently modified plants in research because of the well developed transformation method, well studied genomes and easy propagation (Koornneef and Meinke 2010). They also serve as model organisms for other plant species.

TYPES OF MODIFICATIONS

Three types of modifications are met with. These are transgenic, cisgenic and subgenic. Transgenic plants have genes inserted into them that are derived from another species. The inserted genes may be from plant to plant or from bacteria to plant. In order to correctly

and efficiently express in the host plant, the inserted DNA has to be modified slightly. Transgenic plants are used to express proteins like the cry toxins from *B. thuringiensis*, herbicides resistant genes, antibodies and antigens for vaccinations (Walmsley and Arntzen 2000). Transgenic plants have been modified to increase photosynthesis (currently about 2% at most plant to the theoretically potential of 9-10%). This is possible by changing the rubisco enzyme (i.e. changing c^3 plants into c^4 plants), by placing the rubisco in a carboxysome by adding CO_2 pumps into the cell wall, by changing the leaf form/ size. Transgenic plants have been developed to exhibit bioluminescence may become a sustainable alternative to electric lighting. Transgenic plants have been modified to fix ambient nitrogen.

Cisgenic plant are developed using genes found within the same species or a closely related one, where conventional breeding may occur. Some breeders are of the opinion that cisgenic modifications are much more useful for plants that are difficult to cross bred by conventional methods, such as in potatoes, and plant is the cisgenic category might not require the same regulatory scrutiny as in transgenic (Mackenzie, 2008).

Gao Caixia, a Chinese researcher claimed development of powdery mildew resistant variety of wheat in 2014. The strain lacks gene that encode proteins that represss defenses against the mildew. All three copies of the genes have been deleted from the wheat's hexaploid genome. The strain so developed is to reduce or eliminate the heavy use of fungicide to control the disease. Gao used the TALENs and CRISPR gene editing tools without changing any other genes (Talbot, 2014; Wang, 2014). Such modification is known as subgenic.

Traits covered under GM Research

The majority of plant biotechnology research so far has focussed on development of plants that resist insects, diseases, frost damages and tolerate certain herbicides. Besides, GM crops grown to day or under development have been modified with various characters which include improved shelf life, production of useful goods such as biofuel or drugs and ability to absorb toxins and for use in bioremediation of pollution. Research and development has also been targetted to enhancement of crops locally important in developing countries, such as insect resistant cowpea for Africa and insect resistant brinjal (Wikipidia). Broadly, the GM research on crops covers the broad areas like, Lifetime, Nutrition, Stress resistance, Pest reistance, Byproducts, Biofuel, Industrial products, Bioremediation and Asexual reproduction.

An idea of the traits on which GM research are directed may be obtained from the table 1.

Table 1

Traits covered under GM research

Traits for improvement	Crops considered
Longer self life	Tomato, Potato, Arctic Apples, Papaya, Mango
Vitamin enrichment	Rice, Corn
Nutrition quality	Rice, Mustard, Potato
Cooking quality	Rice
Edible oil	Soyabean, Coconut, Camelina
Toxin reduction	Cassava, Potato
Abiotic stress tolerance	Rice, Sorghum, Wheat, Brassica
Herbicide tolerance	Blackgram, Cotton, Mustard, Maize, Soyabean
Pest resistance	Tobacco, Corn, Rice, Papaya, Potato, Squash, Cotton
Fungal resistance	Jute, Potato, Rapeseed, Black Gram, Cauliflower, Chickpea, Chilli, Egg plant, Pigeon pea, Rice, Melon, Winged bean
Bacteria resistance	Jute, Wheat, Rice, Tomato
Virus resistance	Papaya, Cucumber, Tomato, Peeper, Cassava, Cucurbits, Yard long beans
Pollution control	Rapeseed/Mustard, Cauliflower
Fibre control	Jutre
Wood quality	Teak
Yield	Oil palm
Biofuel	Alga, Jatropha

The majority of GM technology research in crop plants so far has focussed on development of plants that resist insects, disease, frost damage and tolerate certain herbicides. Insect resistance research has centered on inserting a gene obtained from the bacteria *Bacillus thuruingiensis* (Bt) into plants and bacteria (Kung, 1993). The gene produces a toxin that is fatal to insects. The development of crop varieties tolerant to abiotic stresses like drought, frost, soil salinity and nitrogen starvation through GM technology research are in progress. Drought Gard Maize was the first drought resistant Gm crop which received U.S marketing approval (Eisentein, 2013).

During the later part of the nineties of the preceding century. GM crop research was directed more towards development of glyphosphate resistant crop variety. Glyphosphate, an active ingradient of some herbicides that kills plants. The traits are developed because the herbicides used on grain and grass crop were highly toxic and not effective against narrow leaved weeds. Developing crops that could withstand spraying with glyphosate would reduce both environmental and health risk, besides an agricultural edge to the farmer (Carpenter and Gianessi, 1999) The factor resistant to glyphosphate was isolated from an *Agrobacterium* strain and was engineered for plant expression in Soybean. Tobbaco plants have been engineered to be resistant to the bromoxynil (Debora, 1994). The herbicide glufosinate resistant crops have also been commercialized (Gianessi *et al*., 2002).

Tobacco, corn, rice and other crops have been engineered to express genes encoding for insecticidal proteins from *B. thuringiensis* (Vaeck *et al.*, 1987). Papaya, potatoes and suuash have been developed to resist viral pathogens such as cucumber mosaic virus. Virus resistant papaya were developed in response to a papaya ringspot virus outbreak in Hawaii in the late 1990s.

Jatropha and maize have been genetically modified to convert starch to sugar for biofuel ethanol. Poplar trees have been genetically engineered to contain less lignin to facilitate conversion into ethanol (Hope, 2013). Labs are working on genetic modifications of plants to be used for making bioplastic. Oilseed can be modified to produce fatty acids for detergents, substitute fuels and petrochemicals. A weed *Arabidopsis thaliana* containing genes from bacteria has been developed for cleaning TNT and FDX explosive soil contaminants for bioremediation of contaminated soils with mercury, selenium and organic pollutants e.g. polychlorinated biphenyls (Chard, 2011; Meagher, 2000).

Genetically modified crop plants offer a good scope for asexual reproduction which would retain desirable traits and developed in such crops which otherwise reproduce through sexual methods only. For example, crops like maize which reproduces sexually each year, undergoes randomization of genes when propagated to next generation through sexual reproduction. This means desirable traits developed in a generation can be lost in the next generation and there are no alternatives to the farmers but to go for purchase of seeds every year to maintain a high standard crop. High cost of such seeds also effects a majority of farmers. The seed companies maintain two inbred varieties crosses them into a hybrid strain that is sold at high price. Apomixis, a form of asexual reproduction that keeps the plants DNA intact might be useful for sorghum and gammagrass but traditional breeding has been unsuccessful in creating asexually reproducing maize. The path of genetic engineering has led to successful modification of maize crop to sow harvested seeds retaining desirable traits in the crop ensuring asexual reproduction.

Application

The first transgenic plants were engineered in the 1980s. Hall, Kemp and co-workers transferred the â-Phaseolin gene from bean to sunflower and tobacco plants (Murai *et al*.,1983). It was only in 1983, after the discovery of DNA structure, first foreign genes from the prokaryotic bacterial cell *Escherichia coli* were inserted into petunia and tobacco (Kung, 1989).Thereafter several transgenic tobacco plants were produced independently to express foreign genes engineered by *Agrobacterium tumefaciens* vectors (Murai *et al.,* 1984; Horsch *et. al*., 1984 and De Block *et. al.,* 1984). Break throughs in the regeneration of the monocot plants rice and maize have led to improving cereal crops.

Biotechnologists are engaged in the development of transgenic rice since long and there are already genetically engineered rice with desirable characters. Golden rice (vitamin A rich rice) each an example. Golden rice has been produced through genetic engineering to biosynthesize beta carotene, a precursor of vitamin A(Ye et.al,2000; Potrykus,2001).It was developed through incorporation of Psy (phytoene synthase) gene for the enzyme from daffodils (*Narcissus pseudonarcissus*) and that of Crtl enzymes from the soil bacterium *Erwinia uredevora*, Insect-pest resistant Bt.-rice is another example. Recently, a group of Japanese scientists transferred a gene with CPY 2B6 enzyme code from human liver to rice plant resulting in development of rice varieties which is resistant to 13 herbicides

(http..// news.independent.co.uk/world/science technology). With the recent discovery(2005) of genetic code of rice and existence of 37,544 genes in rice genomes, under International Rice Genome Sequencing Project, the possibilities of development of rice varieties with increased nutrition value, insect- pest resistance and high yield have further brightened (*http*://news.enet.com/8301_10784_3_5827461_7.html). August 10, 2005.

A good number of food crops which have been genetically modified inrespect of some traits, are now available for commercial cultivation. A list of such varieties along with their characters modified are given in table 2.

Adoption of GM crops

The first commercially grown GM food crop was a tomato created by California Company in the early

Table 2

Available genetically modified food crops for commercial cultivation

Crops	Traits modified	Modifications achieved through
Alfa alfa	Tolerance of glyphosphate, glufosinate	Genes added
Apples	Delayed browning	Genes added for reduced polyphenol oxidase (PPO). production from other apples
Canola/Rape seed	Tolerance of glyphosphate or glufosinate. High laurate canola, Oleic acid canola	Genes added
Corn	Tolerance of herbicide glyphosphate or glufosinate, and 2,4-D.insect resistant. Added enzyme, that converts starch into sugar to facilitate ethanol production.	Genes added
Cotton (Cotton seed oil)	Insect resistance	Genes, some from Bt. added.
Egg plant	Insect resistance	Genes, some from Bt. added.
Papaya (Hawaiian)	Resistance to the papaya rings for virus	Genes added
Potato (Food)	Resistance to beetle, potato leaf roll virus, potato virus Y; Reduced acrylamaide when fried and reduced bruising.	Bt. Cry 3A,coat protein from PVY "Innate" potatoes added genetic material coding for mRNA for RNA interferance
Poatato starch	Antibiotic resistance, Better starch production	Antibiotic resistance gene from bacteria addedModifications to endogenous starch producing enzymes
Rice	Enriched with beta-carotene (a vitamin A precursor)	Gene from maize and a common soil micro organisms
Soybean	Glyphosphate or glufosinate tolerance, Reduced saturated fats oleic acid	Herbicide resistant gene from bacteria Removal of native genes that catalyse saturation
Squash(Zucchini/ Courgette)	Resistance to water melon and Zucchini/Courgette yellow mosaic virus	Added viral coat protein genes
Sugarbeet	Glyphosphate, glufosinate tolerance	Added genes
Sugarcane	Pesticide tolerance High sucrose content	Genes added
Sweet peppers	Resistance to cucumber mosaic virus	Viral coat proteins
Tomatoes	Suppression of the polygalacturonase enzyme retarding fruit softening after harvesting and retaining natural colour and flavour of the fruit.	Antisense gene of the gene responsible for PG enzyme production added

Source: "From Wikipedia", the free encyclopedia, Genetically modified crops

1990s. Subsequently, in 1996 more GM seeds were planted in United States commercially. In 2013, GM crops were grown in 27 countries of which 19 were developing and 8 were developed countries. In 2013, the total area cultivated with GM increased by 100% to 175.2million hectares. 10% of the world's crop land were protected with GM crop in 2010. Growing GM crops commercially was most prevalent in the USA, Brazil, Argentina, India, China, Canada, Paraguay, Pakistan, South Africa, Uruguay, Bolivia, Australia, Philippines, Myanmar, Burkina, Faso, Mexico and Spain. Europe grows few genetically engineered crops with the exception of Spain where 20% of maize is genetically engineered. The European Union had a "de facto" ban on the approval of new GM crops from 1994 to 2004.

China is the only country in Asia growing a significant amount of GM crops. China claims to have developed the first genetically modified wheat in 1996. The country is the largest importer of soybean. India, however, rejected imports of GM corn-soya blend in 2002 (John Fetter, 2004).

In the Asia-Pacific region, four countries have GM crops under commercial cultivation. In Australia, approximately 0.1 million hectare are under insect resistant and HT cotton (James, 2007). GM Canola and GM carnation have also been approved for environmental release in Australia. China has been cultivating Bt. cotton since long, the area under the crop reached 3.8 million hectare in 2007. In India Bt. cotton occupied an area of 6.2 million hectare in 2007. Sixty two Bt. cotton hybrids were released by the end of 2006, their number increased to 156 by May, 2008 (IGMORIS, 2008a). Bt. Maize in Philippines were grown for the first time in 2003, covered an area of 0.3 million hectare in 2007. Recently cotton and maize with "stacked gene" combining insect resistance with herbicide tolerance have been released (Gupta *et al.*, 2008).

Maize, soybean, cotton, canola, sugarbeet, alfalfa, squash account for almost all GM crop production over the world, the total planted area under which registered at 175.2 million in the world during 2013. USA accounted for the highest coverage followed by Brazil and Argentina as may be evident from the table 3.

Benefits from GM crops

Economic impacts of GM crops have been studied on Bt-cotton in China and India and Bt-corn in the Philippines. Huang *et al.* (2002 a, 2002 b) reported that on an average Bt-cotton yield increased by 8% to 15% higher than non-Bt-cotton in China. Simultaneously, there was a cost saving in pesticide application which reduces cost of production up to 33% Dong et. al. (2004) reported that, true breeding Bt-cotton varieties resulted incremental benefits by reducing pesticide use, reducing environmental pollution and saving labour. However, the increased income was due to cost saving and not than increase in yield. Hybrid BT-cotton, however, resulted in 20% higher yield over BT-cotton varieties. Qaim and Zilbermann (2003) reported, on an average, BT-hybrids received three times less sprays against bollworm for non Bt-hybrids and local checks in India. Bennet *et al.*, (2009) also observed under Indian condition that the number of sprays received for bollworm control was much lower for Bt-plots resulting in 83% reduction in cost.

In the, Philippines, Ebora *et al.* (2005) observed Bt-maize gave a 34% higher yield than non Bt-maize and there was a saving in insecticidal spray.

While analysing the environmental impact of GM crop cultivation that resulted from changes in insectides and herbicides uses, Brook and Barfoot (2006) observed that GM crops contributed significantly to reduction in global environmental impact on production in agriculture by reducing the use of pesticide active ingredients by 6.9% and the overall environment impact associated with pesticides use by 15.3%.

Table 3

World coverage under GM crops

Country	Coverage 2013 (million hectare)	GM crops grown
USA	70.1	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash
Brazil	40.3	Soybean, maize, cotton
Argentina	24.4	Soybean, maize, cotton
India	11.0	Cotton
Canada	10.8	Canola, maize, soybean, sugarbeet
Total	175.2	–

Source: Wikipedia

Arunachalam and Bala Ravi (2003), Sahai (2005) observed, that not all the studies, however, have reported positive impact of GM crops. Some of the surveys on BT-cotton in India have reported lower yield than conventional cotton, particularly, under rainfed condition and lower net returns to farmers (Sahai and Rahaman, 2003; Qayun and Sakhari, 2005).

Risk factors

Commercial production of GM crops are faced with a number of risk factors, the most important being safety on environment and human and animal health (Grumet and Gifford, 1998; Khetarpal, 2002 and Philippe, 2007). A brief discussion on the risk issues of application of GM technology in agriculture may be made as below.

Gene flow in transgenic crops is a serious concern as it reverses the efforts to contain transgenes and posses challenges in assessment and management of risk that are associated with GM crops (Conners *et al.*, 2003; Nap *et al.*, 2003). Risks with transgene flow may lead to development of new weeds, erosion of genetic diversity in wild and weed relatives of crop plants and development of abiotic resistance in pathogenic micro-organisms. Besides genetic erosion, commercialization of transgenic crops in centres of diversity could lead to a pollution of gene pools which is major concern for gene bank management (Engeis *et al.*, 2006; APCOAB, 2006). Horizontal gene flow could result in transfer of transgene to unrelated organisms effecting environment and human health (Netherwood *et al.*, 2004). Antibiotic resistant genes, used as markers during development of GMOs are particular concern since of their transfer to plant or human pathogenic bacteria could lead to development antibiotic resistant strains.

The introduction of new protein in transgenic crops from the organism that are not consumed may pose the risk of those proteins becoming toxic or allergic (Cockburn, 2002; Goodman *et al.*, 2005). Safety of food products derived from GM crops with Bt protein as also with new toxins under development and testing has been a major area of concern (Heinemann, 2007).

The toxin produced by genetically engineered insect pest resistant plants may have adverse effect on non-target insect pest associated with such plants (Hilbeck *et al.*, 1998). Disease or pest resistance developed by a transgene may become ineffective in the event of evolution of new pest strains. Some recent reports on insect developing resistance to Bt. toxins are already there (Tabasnik, 2008).

There are apprehensious that dominance of one or a few GM varieties/hybrids may result in disappearance of traditional varieties as happened with the large scale adoption of high yielding crop varieties after green revolution (Basu and Majumdar, 2010).

Gene silencing i.e. partial or complete inactivation of a trans gene or its homologus gene in the recipient plant has been reported in several experiments (Cherdeshewasart *et al* 1993; Dunwell, 1999).

The development of " Terminator Technology" and its use in the process of production of seeds of trans gene crop is another problem faced by the growers in raising treansgenic crops (Banerjee and Basu, 2000). The term "Terminator seed" has been applied to those which would give a normal crop in one season, but the saved seeds from the crop will fail to germinate for the next crop. To prevent transfer of transgenes preserved in the transgenic crop plants to a normal crop variety through normal – back crossing, the inventors of transgenic crop might go for adoption of the "Terminator Technology", to protect their interest and authority in the production of transgenic crop seeds, which would in effect prevent farmers from using saved seeds, consequently forcing them to procure costly fresh seeds from the company every year. The in-built terminator will fully prevent the use of seeds from transgenic crops for the next season. The terminator technology when applied to either a transgenic or pure lines or a normal HYV will protect the desired transgene(s)/ traits from being pirated by a plant breeder elsewhere since with the use of the technology, back cross seeds will not germinate for the next crop (Banerjee and Basu, 2000; Basu and Majumdar, 2010).

CONCLUSION

With the advancement of the genetic engineering techniques, it has been possible to adding or removing genes from the crops in order to develop a crop with specific traits. Recombinant DNA based GM technology differs from conventional breeding in that totally new genes are transferred between widely unrelated organisms, having certain specific traits of economic interest. GM crops have been developed that resist insects, disease, frost damages and tolerant to certain herbicides, yield and product quality. Besides, GM crops have been developed with improved shelf life, production of useful goods, such as bio-fuel or drugs, lifetime nutrition, cooking quality etc. The crop being modified, include cereals, pulses, oil seeds, vegetables, fiber crops, fruits and several others in respect of the aforesaid traits.

However, the effects of GM crops on health, environment, crop diversity and their impact on farmers

vis-à-vis the role of the seed producing companies cannot be ignored. Basic information on several bio-safety uses, especially food safety and environmental effect of GM crops have to be adequately addressed without which wide spread use of this technology may not materialize.

REFERENCES

APCOAB. 2006. Bt. Cotton in India- A status report. Asia Pacific Consortium Agricultural Biotechnology, New Delhi, India, 34.

Arunachalam, V. and S. Bala Ravi 2003. Concieved conclusions in favours of GM cotton? A riposte to a paper in Science. *Current Science,* **85,** 1117-1119.

Banerjee, S.P. and Basu, N.C. 2000.Transgenesis to terminator technology – An overview. *SATSA Mukhopatra- Annual Technical issue*, West Bengal, **4**, 1-8.

Basu, N.C. and Majumder, M.K. 2010. Genetically modified crops- Benefits and risk issues. *Indian Biologist*, special volume, 68-79.

Bennet, R.M., Ismeal, Y., Kambhapati, V. and Morse, 2000, 2004. Economic impacts of genetically modified cotton in India. *Ag. Bio. Forum*. **7,** 96-100.

Bock, R. 2010. " The give and take of DNA: horizontal gene transfer in plants". *Trends in Plant Science*. **15** (2), 11-22.

Brook, G. and Barfoot, P. 2006. Global impact of biotech crops :Socio Economic and environmental effects in first ten years of commercial use. *Ag. Bio. Forum*. **9**; 139-151

Carpenter, J. and Gianessi, L.1999. Herbicide tolerant soybeans, why growers are adopting Roundup Ready varieties. *Ag. Bio. Forum*, **2**(2), 65-72.

Catchpole,G.S., Beckman., M, enot, P.P., Mondha, M., taylor, J., Hardy, N., Smith, A., King, R.D., Kell, D.B., Fiehn, O., Draper, J. 2005. Hierarchical metabolomics demonstrates substantial compositional similarity between genetically modified and conventional potato crops. *Proceedings of the National Academy of Sciences*. **102** (40): 14458-14462.

Chard, Abegail. 2011. Growing a grass that loves bombs. The British Science Associastion, September, 2011.

Chen, Z. 2010. Molecular mechanisms of polyploidy and hybrid vigour. *Trends in Plant Science*. **15**(2), 57-71.

Cherdeshewasart, W., Gharti, G.B., Chhetri, M.W., Soul, M.J. and Negrutiu, I. 1993. Expression instability and genetic disorders in transgenic *Nicotiana plumbaginifolia* L. plants. *Transgenic Research* **2**, 307-320.

Cockbern, A. 2002. Assuring the safety of genetically modified (GM) foods: The importance of a holistic integratic approach. *Journal of Biotechnology*, **28**, 79-106.

Conners, A.J, Glave, T.R. and Nap. J. 2003. The release of genetically modified crops into the environment: Part II. Over view of Ecological Risk Assessment. *The Plant Journal*, **33**, 19-46.

Cordaux, R., Udit, s., Batzer, M., Fesshotte, C. 2006. Birth of Chimeric primate gene by capture of the transposase gene from a molecular element. *Proceedings of the National Academy of Sciences of the United States of Americ,* **103**(22), 8101-8106.

De Block, M., Herrera-Estrella, Van Montagu, Schell, M.J. and Zambroyski, P. 1984. *Embo J*. **3,** 681-686.

Debora, M. 1994. Transgenic tobacco is European first. *New scientist*.

Dong, H., Li, w., Tang, W. and Zhang, D. 2004. Development of hybrid Bt cotton in China- A successful integration of transgenic technology and conventional techniques. *Current Science*, **86,** 778-782.

Duncan, R. 1996. *Tissue Culture- induced variation and Crop Improvement*, **58**.

Dunwell, J.M. 1999. Transgenic Crops : The next generation or an example of 2020 vision. *Annals of Botany*, **84**, 269-272.

Ebora, R.V., Ampil, A.G., Palapac, M.B. and Custodio, C.G. 2005. Commercialization of Bt-corn in the Philippines- *A status report. Asia Pacific Consortium on Agricultural Biotechnology*, New Delhi, India, 32.

Eisenstein, M. 2013. Plant breeding: Discovery in a dry spell. *Nature,* **501**, 57-59.

Engels, J.M.M., Ebert, A.W., Thoruman, I. and de Vicente, M.C. 2006. Centres of crop diversity and/or origin, genetically modified crops and implications for plant genetic resources conservation. *Genetic Resources and Crop evolution*, **53**, 675-1638.

Feschotte, C., Osterlund, M.T., Peeler, R. and Wessler, S.R. 2005. DNA binding specificity of rice mariner-like transposases and interactions stowaway Mites. *Nucleic Acids Research,* **33** (7), 2153-65.

Gianessi, L.P., Silvers, C.S., Sankura, S. and Carpenter, J.E. 2002. Plant Biotechnology: Current and potential impacts for improving pest management in U.S. Agriculture, An analysis of 40 case studies (Washington, D.C.: National Centre for Food and agricultural Policy), 5-6.

Goodman, R.E., Hefle, S.L. and Reo, R.V. 2005. Assessing genetically modified crops to minimize the risk of increased food allergy: a review. *International Archives of Allergy and Immunology,* **137**, 156-166.

Grumet, R. and Gifford, F. 1998. Plant Biotechnology in the United States: issues and chailenges *en route* to commercial production. *Hort Science,* **33,** 701-726.

Gupta, K., Karihaloo, J.L. and Khetarpal, R.K. 2008. Biosafely Regulations of Asia-Pacific Countries. *National Bureau of Plant Genetic Resources*, *New Delhi. Asia-Pacific Consortium on Agricultural Biotechnology*, *new-Delhi* (APCOAB), FAO.

Heinemann, J. 2007. A typology of the effects of (trans) gene flow on the conservation and sustainable use of genetic resources. Background study paper no. 35. FAO.Rome,100.

Hilbecle, A.M., Baumgatruey, P.M. and Bigler, F. 1998. Effect of transgenic *Bacillus thuringiensis* corn fed prey on mortality and development of immature Chryosoperia carnea (Neuroptera; Chrysopidae). *Environmental Entomology*, **27**, 1-8.

Hope, A. 2013. News in brief; The Bio Safety Council …… Flanders Today.

Horsch, R. B., Fraley, R. T., Rogers, S. E., Sanders, F. R., Lloyd, A., Hoffman, M. 1984. *Science*, **223**, 496-498.

Huang, J., Hu, R., Fan, C., Pray, C. E. and Rozelle, S. 2002a. Dt cotton benefits, cost and impact in China. *Ag. Bio. Forum*, **5**, 153-166.

Huang, J., Rozelle, S., Pray, C. E. and Wang, Q. 2002b. Plant Biotechnology in the developing world; *The Case of China Science*, **295**, 674-677.

IGMORIS. 2008B. Transgenic crops under development and field trials in 2006. (*http:// igmoris.nic.in/ field_trails.asp*).

Jackson, D. A., Symon, R. H. and Berg, P. 1972. *Proc. Natl Acad. Sci., USA*, **69**, 2904-2909.

James, C. 2007. Global Status of commercialized biotech/ GM crops: 2007. ISAAA Brief No. 37, ISAAA, Ithaca, N. Y., 125.

John, F., 2004. The future of GM food. The Sunday Statesman, Kolkata, 5 December, 2004. Reprinted from Yale Global Online, (*http:// yale global. yale.edu.*).\

Khetarpal, R.K. 2002. Biosafety of genetically modified crops and its implication. In : Hitech Horticulture: (eds. K.L. Chadha, M.L. Choudhary and K.V. Prasad), *Horticulture Society of India.* New Delhi, 444-453.

Koornneef, M., Meinke, D., 2010. The development of *Arabidopsis* as a model plant. *The Plant Journal: For Cell and Moleculer Biology*, **61**(6), 909-921.

Kung, S. D. 1984. CRC Critical Rev. in Plant. Sci., **I**, 227-267.

Lederberg, J. and Tatum, E.L. 1946. Gene recombination in E. coli. *Nature*, **158** (4016).

Long, M., Betrah, E., Thorntorn, K. and Wang, W. 2003. The origin of new genes: Glimpses from the young and old. *Nature reviews. Genetics*, **4**(11), 865-875.

Meagher, R. B. 2000. Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology,* **3** (2), 152-162.

Meynell, G. G. 1973. Bacterial Plasmids, MIT Press, Cambridge.

Morgante, M., Brunner, S., Pea, G., Fengler, K., Zuccolo, A. and Rafalski, A. 2005. Gene duplication and exon shuffling by helitron like transposons generate intraspecific diversity in maize. *Nature Genetics*, **37**(9), 997-1002.

Murai, N., Sutton, D.W., Murray, M.G., Slighton, J.L., Merio, D.J., Reichert, W. A., Sengupta, G., Stock, C. A., Barker, R.F., kemp, J. D. and Hall, T. C.1983. Science, **22**, 476-482.

Murai, N., Suffon, D.W., Murray, M.G., Slighton, J.L., Merio, D.J., Reichert, N.A., Sengupta, Gopalan, G., Stock, C.A., Barker, R.f., Kemp, J.D. and Hall, T.C. 1983. *Science*, **22,** 0476-482.

Netherwood, T., Martin, S.M., Orue, A.G., O'Donnell, S., Gockling, J., Graham, J.G., Mathers, and Gilbert, H.J. 2008. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature Biotechnology*, **22,** 204-209.

Nop, J.L., Peter, J., Metz, M.E. and Anthony, J.C. 2003. The release of genetically modified crops into the environment Part 1. Overview of current status and regulations. *The Plant Journal,* **33**, 1-18.

Philippe, V. 2007. Trends in GM crop, food and feed safety literature. *Nature Biotechnology*, **25**, 624.

Potrykus, I. 2001. Golden Rice and Beyond. *Plant Physiology,* **125,** 1157-11641.

Predieri, S. 2001. Mutation induction and tissue culture in improving fruits. *Plant Cell, Tissue and Organ Culture,* **64,** 213.

Qaim, M. and Zilbermann, D. 2003. Yield effects of genetically modified crops in developing countries. Science 299:900-902.

Qayum, A. and Sakkhari, 2005. Bt cotton in Andhra Pradesh-A three year assessments Deccan Development Society, A. P. P., 42.

Sahai, S. 2005. The Scienceof Bt. cotton failure in India. The Hindu, 29th July, 2005.

Sahai, S. and Rahman, S. 2003. Study reveals that Bt. cotton performing poorly in India (*http:// www.genecampaign. org/publication/Article/ BA%I20 Cotton/Bt cotton in failure. Pdf/: accesseu on 20 March, 2008*).

Shrawat, A. and Lorz, H. 2006. Agrobacterium- mediated transformation of cereals: a promising approach crossing barriers. *Plant biotechnology Journal*, **4**(6), 575-603.

Tabsashnik, B.E., Cassmann, D.W. Crowden and Carriere, Y. 2008. Insect resistance to Bt. crops: evidence versus theory. *Nature Biotechnology*, **26**, 199-202.

Talbat, David 2014. Beijing Researchers Use Gene Editing to create Disease- Resistant Wheat (MIT Technology Review. Technology review.com. Retrieved 2014-07.

Vaeck, M. *et al.* 1987. Transgenic plants protected from insect attack. *Nature*, **328**, 33-37.

Wang, Yanpen 2014. Simultaneous editing of thace homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature biotechnology*. doi: 10. 1038/nb7, 2969.

Warson, J.D. and Crick, F.H.C. 19563. *Nature*, **171**, 964-967.

Ye X, Al- babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P., and Potrykus, I. 2000. Engineering the provitamin A (beta- carotene) biosynthetic pathway (carotenoid free) into rice endosperm. *Science*, **287** (5451), 303-305.

(Available from http:// en.wikipedia.org/wild/ Golden rice accused on 16.01.10).

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EFFICACY OF BOTANICALS, PHARMACEUTICAL FORMULATION AND WET TREATMENTS FOR EXTENDED STORABILITY, ENZYME ACTIVITY AND IMPROVED FIELD PERFORMANCE OF FIELD PEA (*Pisum Sativum* L., CV. RACHNA) SEED

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ABSTRACT

Studies were conducted to evaluate the effect of dry dressing treatments with different chemicals, pharmaceutical products and crude plant materials and wet trteatments on greminability, different biochemical parameters and field performances of field pea. Treated along with untreated seeds were subjected to natural ageing for three months in cloth bag under ambient condition. Morphological test conducted after three months of natural ageing revealed that dry dressing of seed with red chilli powder @ 1g/kg of seed, amla fruit powder @ 2g/ kg of seed and iodinated calcium carbonate @ 2g/ kg of seed showed significantly higher germination percentage and vigour as measured by root and shoot length of the seedlings over control. Field performance in terms of germination count, 1000 seed weight and yield of dry dressed bottled stored (8 months) seeds especially with red chilli powder and amla fruit powder were significantly higher than the control. Most of the treated seeds showed lower leakage of sugar along with higher enzymatic activity (peroxidase and catalase) than control. Total soluble seed protein banding pattern of different treated and untreated seed lot revealed that there has been reduction in band intensity, band number or even loss of some bands with the progress of ageing.

INTRODUCTION

Field pea (*Pisum sativum* L.) is a popular pulse crop of India. It is a cool season crop requires an optimum mean temperature of 13ºC to 18ºC for its growth. Pea seeds are good source of protein. The crop is grown for their succulent or dry seeds. Peas are used as fresh or processed. The processed form include freezing, canning and hydrated. Mature seed may be used as dal and prepared in various ways for human consumption. Field pea contributes 3 % in total pulse area and about 5% in the total pulse production of India. Uttarpradesh is the major field pea growing state in India followed by Madhyapradesh and Bihar. One of the major bottleneck in increasing the area of field pea in India is the lack of availability of good quality seed. Due to inadequate storage facilities, the farmers are forced to use the deteriorated seed for sowing in the next season causing reduction in yield. Several workers have applied different dry and wet treatments on the seed prior to storage to minimize the deterioration of seed during storage. Pre-storage dry dressing treatments and mid-storage hydration-dehydration treatments of stored orthodox seeds of wheat, soybean and sesamum has been reported to be very effective for maintenance of vigour, viability and productivity (Mandal and Basu , 1983 ; Mandal *et al.*, 2000; 2008)

Pati *et al.* (2011) demonstrated that the pre-treatment of pea seeds with leaf extract of bel (*Aegel marmelos*) and kalmegh (*Andrographis panniculata*) 50 g in 250 ml of distilled water for six hours before accelerated ageing treatment for (100% relative humidity and 30 ± 2ºC temperature) 45 days found much better plant performance than untreated control.

More recently infusion of fungicides, growth regulators, pesticides, bio-products, bio-ingredients, agro-chemicals and herbicides into the seeds prior to germination is reported to alleviate the impact of adverse factors on seed quality and performance (Janmohammadi *et al.,* 2008).

Present study was taken up with a view to development of some suitable seed invigoration treatments so that storability and field performance could be maintained keeping the atmospheric condition of eastern zone in mind.

MATERIALS AND METHODS

Seed Material

To carryout the present experiment, properly dried seeds of current season were collected from the Agricultural Experimental Farm of Calcutta University and were stored in the 2.5 lit. capacity rubber stoppered glass bottle. Pre-storage treatments were given to one month old seeds.

Method of pre storage seed treatment:

a) *Dry treatment :* Dry seed treatment was employed using crude plant materials like red chilli powder (active ingredient, capsaicin) @ 1 g/kg of seed and amla (active ingredient, phyllemblin) 2 g/kg of seed, chemicals like calcium carbonate @ 2 g/ kg of seed , bleaching powder @ 2 g/kg of seed and pharmaceutical product viz, aspirin @ 50 mg/kg of seed separately with the seeds in the rubber stoppered glass bottle and kept under ambient condition. Treated seeds were gently shaken for seven days for thorough mixing of the materials with seed.

b) *Wet treatment :* Seed priming like soaking in double volume of water for one hour followed by drying back to its original moisture content, moist sand conditioning for 24 hours followed by drying and moist sand conditioning for 24 hours followed by soaking in double volume of water for one hour and then drying for 3-4 days until the seeds regain its original moisture content were also done simultaneously.

After wet treatment, seeds were kept in the perforated paper packet and kept in the dessicator containing fused calcium chloride for 4-5 days to stabilize the moisture content to an uniform level and then kept in the small glass bottle and stored under ambient condition.

Natural Ageing: Treated seeds were placed separately in the perforated paper packets having equal number of holes and kept in a cloth bag before subjecting them to natural ageing for various period under ambient condition to evaluate the efficacy of treatments effect on the viability and vigour of seeds.

Seed performance test:

a) *Germination test :* Germination test were carried out immediately after 10 days of treatment and after three months of natural ageing under ambient condition following the method of Punjabi and Basu (1982) with minor modification employing 400 seeds for each treatment. Data were recorded after germination for 7 days at 20 ± 1°C.

b) Bio-chemical test : Biochemical analysis viz. leaching of sugar, enzyme activity viz. catalase and peroxidase and SDS-PAGE gel of the total soluble protein was carried out to judge the treatment effect on the seed quality.

The leakage of sugar was measured following the method of Mc Cready *et al.* (1950) with minor modifications. Four millilitre of ice cold freshly prepared anthrone reagent (0.2% anthrone in 98% sulphuric acid) was added to 2 ml of pre cooled seed leachate in a hard glass test tube and kept in cold for 30 minutes. The intensity of bluish green colour was measured in systronics spectrophotometer at 580 nm. Catalase activity was measured following the method of Aebi (1984) with minor modification where the decomposition of H_2O_2 was measured by recording the decline in absorbance at 240nm for 3 minute. The reaction mixture contained 50mM phosphate buffer (Ph 7.0), 50mM H_2O_2 and 50 µl of enzyme extract in a measuring tube and volume was makeup to 3ml by adding buffer solution. A mixture without H_2O_2 served as blank. Catalase activity was calculated using mM extinction coefficient for H_2O_2 (39.4mM/cm) expressed as amount of H_2O_2 decomposed/min/g of fresh tissue. Peroxidase activity was estimated as per the protocol mentioned in the text book of Plant Physiology by Ghosh and Mukherjee. Hundred mg of 24 hour imbibed seed tissue was measured and crushed in mortar pestle with 10 ml of phosphate buffer (Ph 7.0). The extract is centrifuged at 10000 rpm for 20 minute and the supernatant was collected for estimation of peroxidase activity. Reaction mixture was prepared in the test tube by taking 5 ml buffer, 1 ml pyro catechol and 1 ml H_2O_2. The reaction was started by addition of 1 ml of enzyme extract. Absorbance was measured at 490nm just after the addition of enzyme with the reaction mixture and then after 3 minutes of incubation. The enzyme activity (Δ Abs.) is expressed in terms of difference in absorbance value per gram per minute.

SDS-PAGE of total soluble protein was carried out by using 12 percent acrylamide gel according to the method prescribed by Laemeli (1970). Hundred mg imbibed tissue was taken in mortar pestle and crushed in 2 ml of buffer containing 1.950 ml buffer and 50 ul betamercepto ethanol. The content was centrifuged at 14000 rpm for 30 minutes. Supernatant so collected was used for protein estimation and 50 µg protein was used

from each sample for loading on to the gel. A constant current of 20 mA was applied until the tracking dye crossed the stacking gel. Then a constant voltage of 80 volt was applied until the tracking dye reaches bottom of the resolving gel. Then the gel was stained using coomassie brilliant blue R 250 overnight and destained using a mixture of 100 ml methanol, 100 ml acetic acid and 800 ml of distilled water until the bands were clearly visible.

Field Experiment :

Treated and untreated seeds of field pea were sown in the field at Agricultural Experimental Farm of Calcutta University, Baruipur, 24 Parganas, West Bengal, during *rabi* season (November – March) using completely randomized block design with three replications for each treatment. After thorough land preparation, the fields were divided into 30 sub-plots, each sub-plots having a size of 6 square meter. Seeds were sown @ 60 kg/ hectare giving a spacing of 30 cm between the row and 15 cm between the plant. The fertilizer dose, N: P_2O_5: K_2O was applied @ 40:40:20 kg/ ha. Data on plant population, seed yield and 1000 seed weight was recorded replication-wise. Statistical analysis of the data obtained from laboratory and field was done following the analysis of variance technique (Fisher, 1948) to judge the treatment effect on storability and field performance. The experiments were carried out in two consecutive years (2013-2014 and 2014-2015).

RESULTS AND DISCUSSION

Germination test conducted immediately after treatment (10 days) implies that there was no significant effect in terms of germinability and vigour between treated and untreated seeds (control). But when the same test was conducted after 3 months of natural ageing, it was found that except soaking drying treated seed, most of the treated seeds showed significantly better results in maintaining the germinability than the untreated control. Among the different treatments, seeds treated with red chili powder and amla fruit powder proved to be the best in terms of germinability and vigour as measured by root and shoot length of the seedlings (Table 1).

Among the pre storage wet treatment, soaking-drying showed negative correlation with the germinability might be due to imbibitions injury in the harvest fresh seed. Soaking-injury in freshly harvested legume seeds is a well established fact, probably due to faster rate of imbibition during initial phase of soaking. Imbibitional injury could be minimize by the use of osmoticum polyethylene glycol as it could reduce the water uptake in the soybean (Woodstock and Tao, 1981, Saha and

Table 1.

Effect of pre-storage seed invigoration treatments for the maintenance of germinability of field pea seed (cv. Rachna) after natural ageing for 90 days under ambient condition (79.5± 2.1 % RH and 29.3± 1°C).

Treatments	Germination		Mean Root Length (mm)	Mean Shoot Length (mm)	Vigour Index
	Percentage	Arc Sin Value			
Control	83	65.88	49	11	3952.8
Red Chili Powder	100	90.0	55	16	6390.0
Bleaching Powder	63	65.88	54	15	4545.7
Soaking Drying	58	49.78	45	13	2887.2
Moist Sand conditioning drying	92	73.15	46	11	4169.5
Moist Sand Condition Soaking Drying	92	73.15	43	15	4242.7
Aspirin	92	73.15	54	13	4901.0
Calcium Carbonate	83	65.88	54	14	4479.8
Iodinated Calcium Carbonate	83	65.88	54	15	4545.7
Amla Powder	100	90.0	55	15	6300.0
LSD at 0.05 P	–	3.848	3.035	3.090	4.72
LSD at 0.01 P	–	5.529	4.360	NS	6.78

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.
Abbreviation : NS = Not Significant
Vigour Index = Mean seedling length x Germination percentage

Basu, 1982). The results of the present study further consolidate the earlier findings.

Field performance of different treated and untreated seeds when scrutinized found that germination count, yield per square meter, 1000 seed weight was significantly higher in all the dry treated seeds than the untreated control (Table 2). Among the nine selected treatments, seed treated with red chilli powder, amla fruit powder and iodinated calcium carbonate experienced higher yield than others including control.

Table 2.

Effect of pre-storage seed invigoration treatments on yield and yield attributes of field pea seed (cv. Rachna)

Treatments	Plant Population /m^2	yield (g/m^2)	1000 seed weight (g)
Control	26	181.09	199
Red Chili Powder	28	282.24	210
Bleaching Powder	26	222.76	204
Soaking Drying	25	179	198
Moist Sand conditioning drying	26	219.32	200
Moist Sand Condition Soaking Drying	27	188.05	199
Aspirin	26	220	201
Calcium Carbonate	25	230.4	192
Iodinated Calcium Carbonate	26	242.81	194
Amla Powder	27	266.97	206
LSD at 0.05 P	0.472	2.133	3.035
LSD at 0.01 P	0.678	3.064	4.360

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.

Table 3.

Effect of pre-storage seed invigoration treatments on the leaching of sugar, catalase and peroxidase enzyme activity immediately after treatment i.e. before ageing condition.

Treatments	Germination (%)	Leaching of sugar (µg glucose equiv./ml)	Catalase activity (n mole/min/g fresh tissue)	Peroxidase activity Δ(Abs/g tissue/min.)
Control	100	0.098	4.62	0.288
Red Chili Powder	100	0.095	4.75	0.299
Bleaching Powder	100	0.094	5.10	0.286
Soaking Drying	100	0.106	4.26	0.284
Moist Sand conditioning drying	100	0.099	3.94	0.302
Moist Sand Condition Soaking Drying	100	0.093	4.56	0.294
Aspirin	100	0.092	5.19	0.297
Calcium Carbonate	100	0.090	5.02	0.282
Iodinated Calcium Carbonate	100	0.089	4.87	0.304
Amla Powder	100	0.097	4.97	0.280
LSD at 0.05 P	–	NS	NS	NS
LSD at 0.01 P	–	NS	NS	NS

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.
Abbreviation : NS = Not Significant

The beneficial effect of seed invigoration treatment on improvenment of germinability, storability and field performance of different other crop seeds have been observed by several research worker (Mandal and Basu, 2003, Adebisi *et al*., 2003, Adebisi and Oyekale, 2005). Our present study is also in conformity with the findings of the earlier workers.

LoneIshrat *et al*., (2014) observed that pre-treatment of the maize seeds with chemicals like aspirin , calcium carbonate and crude plant materials like trigonella seed powder and walnut shell powder gave better results on vigour and viability than control.

Biochemical analysis reveals that there was no significant difference between the treated and untreated seeds in terms of leaching of sugar as well as antioxidant enzyme activity viz. peroxidase and catalase when tested immediately after treatment (Table 3). But with the increase in storage period there was a increase in leaching of sugars with simultaneous decrease in enzyme activity (peroxidase and catalase). Most of the treated seeds showed superiority in all the enzyme activity and reduced leakage of sugar over control (Table 4). Among different treatments, red chilli powder, amla fruit powder and iodinated calcium carbonate treated seeds showed significantly lower leaching of sugar and higher peroxidase and catalase enzyme activity than control. Similar decrease in the activity of catalase and peroxidase enzyme was noticed in the aged seeds as compared to fresh seeds (Scialabba *et al.*, 2002, Chauhan *et al.*, 2011). Cakmak *et al*. (2010) also noticed decrease in germination ability of aged legume seeds which was correlated with decrease in enzymatic antioxidant activity. Zamani *et al.* (2010) pointed out that both natural and accelerated ageing reduced germination percentage, seed vigour and activity of catalase, peroxidase and ascorbate peroxidase (APX) with an increase in malondialdehyde (MDA) content and electrolyte leakage in safflower seed.

Non-enzymatic anti-oxidant activity of amla fruit had been confirmed by a number of worker (Kumaran *et al.*, 2006). Amla (active ingredient, phyllemblin) is an effective antioxidant and free radical scavenger helps to reduce disease and slow down the ageing process.

Soluble protein banding pattern of different natural aged (3 months old) seed lot observed a decline in the band intensity, band number or disappearance of some bands as period of ageing advanced. In our present study a total of 19 polypeptide bands of diverse molecular weight ranging from 14.4 KDa to 116.25 KDa was observed. Seed treated with red chilli powder showed maximum peptide bands i.e. 19, while seed treated with soaking-drying had 15 peptide bands and the untreated

Table 4.

Effect of pre-storage seed invigoration treatments on the leaching of sugar, catalase and peroxidase enzyme activity after 90 days of natural ageing under ambient conditions (79.5± 2.1 % RH and 29.3± 1ºC)

Treatments	Germination (%)	Leaching of sugar (µg glucose equiv./ml)	Catalase activity (n mole/min/g fresh tissue)	Peroxidase activity Δ(Abs/g tissue/min.)
Control	83	32.04	1.385	0.136
Red Chili Powder	100	21.19	3.43	0.269
Bleaching Powder	63	25.36	2.64	0.222
Soaking Drying	58	38.67	1.94	0.179
Moist Sand conditioning drying	92	27.4	2.45	0.212
Moist Sand Condition Soaking Drying	92	30.33	2.02	0.204
Aspirin	92	25.34	2.94	0.218
Calcium Carbonate	83	26.41	2.78	0.231
Iodinated Calcium Carbonate	83	23.41	3.01	0.246
Amla Powder	100	22.05	3.58	0.258
LSD at 0.05 P	–	1.48	0.487	0.025
LSD at 0.01 P	–	1.033	0.699	0.037

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1ºC.

control had 15 peptide bands (Fig. 1). Bhanuprakash *et al.* (2006) reported alteration in banding pattern of protein profile of aged seeds compare to that of fresh seed. Vishwanath *et al.* (2007) revealed that due to accelerated ageing there has been a decline in soluble protein banding pattern in terms of band intensity, band numbers or even total loss of some bands.

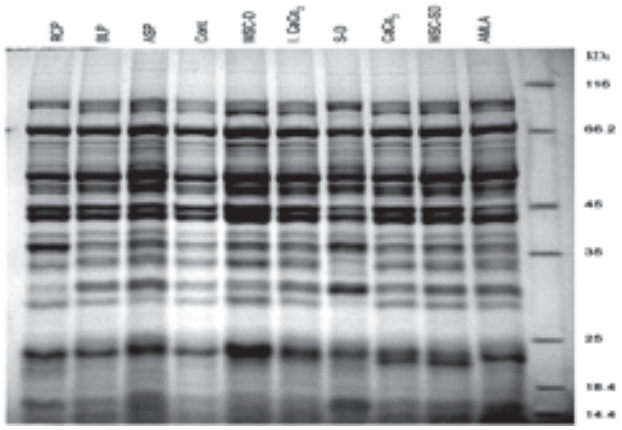

Fig. 1. Peptide profile of different treated and non-treated field pea seeds after 80 days of natural ageing

Abbreviation : RCP- red chilli powder, BLP- bleaching powder, ASP- Aspirin, Cont.- control, MSC-D- Moist sand conditioning drying, I.$CaCO_3$- iodinated calcium carbonate, S-D- soaking drying, $CaCO_3$- calcium carbonate, MSC-SD- moist sand conditioning soaking-drying, AMLA- amla fruit powder

Basu and coworker (Rudrapal and Basu, 1980; Mandal and Basu, 1986) reported that dry permeation of seeds with halogen compounds such as bleaching powder or iodinated calcium carbonate could slow down the deteriorative effect in seed due to ageing. The present study confirm the works of other researchers in this field which establish the fact that dry dressing with crude plant materials like red chilli powder, amla fruit powder and chemicals like aspirin, iodinated calcium carbonate are effective for maintaining the vigour, viability and field performance of different crop seeds (De *et al.*, 2003; Mandal *et al.* 2000; Guha and Mandal, 2013). Hence it is suggested to farmers and seed growers that dry dressing in harvest fresh seeds with red chilli powder @ 1g / kg of seed and amla fruit powder @ 2g/ kg of seed may be practiced for improved storability and field performance of field pea seeds.

REFERENCE

Adebisi , M.A. and Oyekale, K.O.2005.Effect of seed treatments and storage containers on maintenance of viability of okra seed. *ASSET Journal Series* **A5**(1), 81-89.

Adebisi ,M.A., I.O, and Ajala, M.O. 2003. Storage life of soybean seed after seed dressing. *Journal of Tropical Agriculture* (India), **42,** 3-7.

Aebi H. 1984. Catalase in vitro, *Methods in Enzymology,* **105,** 121-126.

Bhanuprakash, K.; Yogeesha, H. S.; Naik, L. B.; Arun, M. N. 2006. Studies on physiological and biochemical changes in relation to seed viability in aged onion seeds. *Journal of Horticultural Sciences*, **1**(1), 15-18.

Cakmak, T., Atici, O., agar, G., and Sunar, S.2010. Natural aging-related biochemical changes in alfalfa (Medicago Sativa L.) seeds stored for 42 years. *International Research Journal of Plant Science*, **1**(1), 001-006.

Chauhan, D.S., Deswal, D.P.,Dahiya, O.S., and Punia, R.C. 2011. Change in storage enzymes activities in natural and accelerated aged seed of wheat (*Triticum aestivum* L.). *Indian journal of Agricultural Sciences,* **81**(11), 1037-1040.

Cakmak T. Atisi O, Agar G. Serap, S. 2010. Natural ageing related bio-chamical change in alfaalfa seed stored for 42 years. *International journal of plant Science*, **1,** 001-6.

De, B.K., Mandal, A.K. and Basu, R.N. 2003. Seed invigoration treatments on different seed sizes of Wheat (*Triticum aestivum* L.) for improved storability and field performance. *Seed Sci. and Technol.*, **31**, 379-388.

Fisher, R.1948. Statistical methods for research workers. Oliver and Boyd, Edinburgh.

Guha, P and Mandal, A.K.2013. Seed treatments for extended storability and their physiology on different vigour status of Okra (*Abelmoschus esculentus* L.), *Indian Agric*., **57**, 29-36.

Janmohammadi M, Moradi D, Sharifzadeh F. 2008. Seed invigoratin techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. Gen. Appl. *Plant physiol*., **34**(3-4), 215-226.

Kumaran A. Larunakanan R.J. 2006. Nitric Oxide radical scavenging active components from *phyllanthus emblica* L. *Plants Foods Heun Nutn*, **61**(1), 1-5.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*., **227**, 680-685.

LoneIshratAhamad, BhatShabir A, SheikhSubzar A and DasM. Suliaman. 2014. Effects of Dry Seed treatments on various quality characters in Maize (Zea mays L.). *Internationaljournal of innovative Science, Engineering & Technology*, **1**(4), 512-521.

Mandal. A.K. and Basu, R.N. 1986. Vigour and viability of wheat se3ed treated with bleaching powder, *Seeds and Farms*, **12**, 36-48.

Mandal , A.K., De, B.K., and Basu, R.N. 2003. Seed invigoration treatment on different seed sizes of wheat (*Triticum aestivum* L.) for improved storability and field performance. *Seed Science and Technology*, **31**, 379-388.

Mandal A.K., De, B.K., Saha, R. and Basu, R.N. 2000. Seed invigoration treatments for improved storability, field emergence and productivity of soybean (*Glycine* max [L.] Merrill). *Seed Sci. and Technol*., **28**, 201-207.

Mandal, A.K. and Basu, R.N. 1983. Maintenance of Vigour, Viability and yield potential of stored wheat seed. *Indian J. Agric*. Sci., **53**, 905-912.

Mandal, A.K., De, B.K., Saha, R. and Basu, R.N. 2000. Seed invigoration treatments for improved storability, field emergence and productivity of soybean (Glycine max (L.)Merrill). *Seed Sci. and Technol.*, **28**, 201-207.

Mandal, A.K., De, B.K.,Chaterjee,B.K. and Kapri,B.2008. Pre-storage dry seed treatments for improved vigour, viability and field performance of sesamum (*Sesamum indicum* L.). *Indian Agric*., **52,** 127-132.

Mc Cready, R. M. , Guggols, J., Silviers, V. and Owen, H.S. 1950. Determination of starch and amylase in vegetables. *Ann. Chem.*, **22**, 1156-1158.

Mukherjee, S. and Ghosh, A.K. 2005. A text book on plant physiology published by *New central book agency (P) Ltd.*, Kolkata, India.

Pati, C.K. and Bhattacharjee, A. 2011. Alleviation of seed deterioration and enhancement of plant potential of a pea species using selected medicinal plant extracts. *Journal of Medicinal Plants Research*, **5**(17), 4108-4111.

Punjabi , B and Basu, R.N. 1982. Testing germination and seedling growth by an inclined glass plate blotter method. *Indian J. Physiol*., **25**, 289-295.

Rudrapal, A.B and Basu, R.N. 1980. Iodine treatment of mungbean seeds for the maintenance of vigour and viability. *Curr. Sci.*, **49**, 319-320.

Scialabba, A., Bellani, L.M. and Dell'aquila, A. 2002. Effects of ageing on peroxidase activity and localization in radish (*Raphanus Sativus* L.) Seeds.*European Journal of Histo Chemistry,* **46**(4), 351-358.

Saha, R. and Basu, R.N.1982. Pre-conditioning soybean seed for viability maintenance by soaking drying treatment. *Seed Research*, **10**, 183-187.

Vishwanath, K., Prasanna, K.P.R., Rajendra Prasad, S., Ramegowda, S. NarayanaSwammy and pallavi, H.M. 2007. Influence of accelerate of ageing on total soluble seed protein profile of tomato. *Seed Research*, **35**(2), 194-197.

Wood Stock , L.W. and Tao, K.J. 1981. Prevention of imbibational injury in low vigour soybean embryonic axes by osmotic control of water uptake. *Physiologia planatarum*, **51**, 133-139.

Zamani, A., Sadat Noori S.A., Tavakkol Afshari, R., Irannejad, H., Akbari, Gh.A., Tavakoli, A. 2010. Lipid peroxidation and antioxidant enzymes activity under natural and accelerated aging in safflower (*Carthamus tinctorius* L.) seed. *Iranian Journal of Field Crop Science* (Iranian Journal of Agricultural sciences), **41**(3), 545 - 554.

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EDITORIAL

We are delighted to publish the Indian Biologist, volume 47, No. 1, 2015 as a special volume on Genetically Modified Crops along with other important aspects within the stipulated date.

We are glad to announce that next two issues will be published as special issues on two burning problems of our environment which affect the very existence of a large number of biological species including human species. Topics of coming two special issues of Indian Biologist are as follows : (i) Pesticides in Agriculture and Horticulture (ii) Prospect and Constraints in Organic Farming.

Usual research papers of our Members on other biological topics will also be included in those special issues.

The Indian Biologist is circulated to all major countries around the world and abstracts of its articles are now being published regularly in leading abstracts of the world in different languages. During the last 47 years, Indian Biologist has gained notable reputation in India and abroad mainly due to the constant patronage from our members and well wishers. The quality of its publication are commendable. We would like to inform our members and research contributors that we have modified the getup of our journal from this issue which will also be beneficial for the higher NAAS ranking. The 'Indian Biologist' has been rated 2.29 by the NAAS academy for the year, 2014 (w.e.f. 1st January, 2015). We are trying hard to improve the quality of the research article as well as printing for further higher rating of our Journal by the NAAS academy. We hope, the authors/ contributors of the research papers or articles will bear us in this regard.

The contributors are cordially requested to send their manuscripts/articles in original for publication in the journal of "Indian Biologist". We will try our level best to publish the issue on scheduled time.

We sincerely request our members and well wishers to offer their patronage and co-operation as before.

T. M. Das and A. K. Mandal