GENETIC ENGINEERING OF INSECTS AND THEIR APPLICATION

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I. INTRODUCTION

Genetic engineering or genetic modification is the applied biotechnology where the entire or some part of the DNA makeup of an organism has been changed in the laboratory using different techniques. The foremost recombinant DNA molecule was synthesized by Paul Berg in 1972, where he combined the DNA molecule from the monkey virus SV40 with the lambda virus. Era of biotechnology was begun in 1973 with the creation of functional bacterial plasmid as the experimental result of Cohen and Boyer under in-vitro condition (Cohen *et al*., 1973). In 1980s for biotechnological uses first GM insect was created (Marx, 1987). Evolution of applied biotechnology has been occurred during last thirty years, in 2009 Food and Drug Administration first approved a factor present in goat milk which prevents clotting, that was first approved pharmaceutical produced by a GM animal (Pollack,2009). Considering the molecular complexity, the GM insect occurred between the pharmaceuticals providing GM animals and GM crops. Before applying of rDNA technology several scientists applied sterile insect technique for managing worst screwworm fly in southern US during 1950-60s (Bushland *et al.*, 1955). Genetic manipulation in insects are carried out by mutagenesis or more precisely can be said that, through transgenesis or cis- genesis with an aim of fulfilling different purposes like food production, oil manufacture, tackling health issues and managing the harmful organism through biotechnology. Trans- genesis in genetically modified insects is carried out by insertion of one or more than one external sequences of DNA particle extracted from foreign organism in to the target insect. The reasons behind the production of GM insects are managing the agriculturally important insect pests, maintaining quality of produce, managing environmental issues and issues concerned with human health and reduction of adverse effect of synthetic pesticides used in pest management systems. GM insects are developed for achieving the goal for managing the insect vectors of human diseases like malaria and dengue fever. It is well proven that modification of insects and other arthropods in the genetic level can be effective strategy for studying the development and evolution at molecular level (Horn and Wimmer, 2000). These genetic modification strategies can be considered as the effective tool for solving several agricultural and health issues which is caused by certain insect. The insects can be altered in the genetic level with suitable desired traits and released in the nature cautiously (Irvin *et al.*, 2004). Utilization of transgenic insects offers a new way to combat the vector insects responsible for spreading human diseases and crop losses due to insect pest. Though the risk assessment and acceptance of the risks associated with the GM insects compared to the potential gain is the main determining factor in the way of developing GM insects.

II. HISTORY OF GENETIC ENGINEERING IN INSECT

Prior to the 1950s, New World Screw-worm *Cochliomyia homnivorax*, became the major health hazarding factor of cattle which was extremely difficult to control. Hypothetical thought of Raymond Bushland and Edward Knipling suggested that if sterilization of male flies is done, then a several female fly can be prevented from egg laying with a help of a single male fly that would eventually shrink the population. Besides this the sexually viable males also would outnumbered by the sterile males. Experimentation for fulfilling these objectives gamma rays, UV rays and ethyl methyl sulphonate were used with an aim of generating the insect population having with a higher sterile to fertile ratio. History of GM insects was marked by the concept of controlling

insect through genetic way given by E. F. Knipling (Joe, 2010). Genetic modification of insects by insertion of one or more DNA particle from foreign sources was first performed in a pest of stored grain namely *Ephestia khuniella* with a mutant line which had no scale in their body. In 1965 when external wild type DNA was introduced in the pest then the transformed adults produced wing scales (Atkinson and James, 2002). Previously genetic change through germ-line transformation was carried out in *Drosophila melanogaster* with the help of transposition by *P* element (Rubin and Spradling, 1982). The gene *rosy*+ was responsible for wild which was successfully inserted in *rosy* mutant *Drosophila* using a vector namely transposable *P* element. In this way wild red eye color got stably introduced in the mutant strain and successfully inherited, though this transformation was restricted in the subgenus *Sophophora* under the genus *Drosophila* but in fewer members (Handler *et al*, 1993). After 13 years later first non-drosophilid insect, that was Mediterranean fruit fly, *Ceratitis capitata*, was transformed by application of a transposable element (*minos*) (Loukeris *et al.*, 1995).

III. NEED FOR GENETIC EENGINEEING IN INSECT

1. Pest management for broad area concerned with health and agricultural importance.
2. Can be used for manufacturing pharma products by using as bioreactors.
3. Development of traits resistant to virus in economically important insects.
4. Enhancement of quality agricultural production, productivity.
5. Beneficial management of public health.
6. Improving the ability to resist diseases, attributes in honey bees regarding pollination andimproving silk worm moth for better quality silk production (Gopinathan, 1992).

IV. STRATEGIES OF GENETIC ENGINEERING IN INSECTS

Production and application of GM insects in different field is based on several strategies which can be classified in the following way-

1. **Self limiting:** The principal goal in the self limiting strategy is population suppression. In this strategy, expectation suggested that the novel characteristics should face more or less rapid disappearance after releasing in the field. So the modified desired trait can be maintained only after releasing the modified additional insects periodically. One of the popular examples of self limiting strategy is the sterile insect technique (SIT) where genetically sterile male is released in the field which is regarded as low risk and least controversial method of genetic control (Camilla *et al.*, 2012). Self-limiting methods also focused on other methods with an aspect of female destruction, sex-ratio alteration and delayed in conditional lethality. (Foster *et al*., 1988; Fryxell & Miller, 1995; Schliekelman & Gould, 2000a; Schliekelman & Gould, 2000b; Schliekelman *et al*., 2005; Bax & Thresher, 2009).

2. **Self sustaining:** Self-sustaining strategies are designed to focus on the modified traitsto be indefinitely persisting in the environment and spread in the native population and increase its frequency and geographical area over time. This strategy mainly focused on control of insect vectors of diseases of human being (Marshall & Taylor, 2009). These techniques aimed for replacing the population of harmful insects into a non-harmful or comparatively harmless mode, as for example it may be a condition with less ability to

transmission of one or more pathogens. The goal can be achieved by transgenesis where a transgene may prevent *Plasmodium* species to infect the vector mosquito. For effective outcome, huge proportion of the population of vector mosquitoes should have this desired gene in a particular area and the transformed species should established in the wild population after release.

V. PHYSICAL METHODS OF GENETIC TRANSFER

1. **Microinjection:** Microinjection was first described by Lin in mouse egg in 1960. It is a physical process of delivering external or foreign DNA inside the cell or egg or oocyte or embryo of an animal. In this method micropipette made of glass is used for injecting the substance containing foreign DNA in a liquid form under the stereomicroscope. Here a Holding pipette is used to hold the particular target cell aimed to insert the foreign DNA of desired trait, the cell is held at its tip when the cell sucked gently. Then micropipette tip is inserted through the cell membrane. In this way the material of the needle is introduced inside the cell cytosol and after that it is withdrawn. The inserted DNA tried to integrate itself with the nuclear DNA of target cell randomly and the expression of this foreign DNA is only possible when it successfully attached with the proper and suitable promoter sequence. Sometimes the contents are also delivered in the intercellular space. This delivery system is generally used for transformation of cells as well as tissues in animals, embryos and eggs by inserting the desired genetic molecule as like DNA, RNA, proteins and macromolecules in a direct way.

2. **Biolistics:** It is also known as particle (heavy metal) mediated gene transfer. Sometimes it is called gene gun or a biolistic gene delivery system, which is mainly devised for delivering foreign DNA into plant cells. In this system the instrument composed of a bombardment chamber, connective tubing for attaching with the vacuum source and other parts like helium regulator, solenoid valve etc. to for attaching and delivery of the helium kept under high pressure, in the main unit. The desired gene (DNA particle) is coated on the particle of heavy metal like gold or tungsten. The particles coated with external DNA are propelled in the target cell at higher velocity and release the DNA particle which integrates itself with the genome of the particular cell.

3. **Lipofection:** It is known as liposome transfection, in this method liposomes are used for transforming a cell by inserting foreign genetic material. Liposomes are small vescicle and capable of merging themselves with the cell membrane as both of them have bilayer made of phospholipid. Positively charged (cationic) liposomes are generally used in lipofection to form a transfection complex with the genetic material with desired traits which is negatively charged (anionic). The complex then fuses with negatively charged cell membrane and get entry inside the cell through endocytosis. Then it goes through the endosomal pathway and releases the foreign genetic material and then the external DNA enter inside the cell genome.

4. **Electroporation:** It refers to the transformation of cell via direct gene transfer action. In this method target cells along with desired DNA are exposed to electrical pulses with very high voltage (4000 – 8000 V/cm) for few milliseconds. As a result transient pores formation in the plasma membrane takes place that can be called as electropermeabilization and by this pores DNA enters inside the target cell and get

integrated with cell nucleus. Success of this process depends on salt concentration. Highersalt concentration may cause electrical discharges. Electroporation is more suitable for those cells that are arrested at metaphase stage as nuclear envelope is absent in this stage as well as unusual permeability of plasma membrane.

VI. DIFFERENT METHODS USED IN GENETIC MODIFICATION OF INSECTS

1. **Transposable elements:** Transposons or transposable elements are a kind of selfish genes and they are pieces of DNA mobile in nature and do not stay in fixed location of a genome (Kidwell & Lisch, 1997; Liao, 2000). The movement of rare sequences of DNA from one position to another positon within the genome of a single cell is often termed as transposition. Through this action they can derive mutations inside the genetic structure. Transposons are also termed as "jumping genes", as they are mobile in nature. First discovery of these jumping genes was done by Barbara McClintock for which she was dignified with Nobel Prize in 1983. Based on the mechanism of their transposition these elements are broadly classified.

    - **Mechanism:** Integration of a transgene by the transposition needed a donor and a helper plasmid; among them no one is autonomous. Desired gene to be integrated and a detectable marker for visual identification are held by the plasmid acting as donor in between its functional terminal inverted repeats (TIR). Being absence or presence of defective TIRs the helper primer cannot start transposition; only function of them is encoding the transpoase enzyme required for transposition. It was also found that using of capped mRNA replacing the helper plasmid provided more effective results in some cases (Pavlopoulos *et al.,* 2004). Several studies showed that microinjection in early embryos provided better result where plasmids are provided in to the germplasm of syncytial blastoderm stage as because the primordial germ cells are formed there and nuclei of some cells taken up the plasmids with gene of interest. After developing as an adult, the concerned transpoase enzyme recognizes the particular TIRs and start transposing the gene of interest that is constructed from donor plasmid on the particular chromosome. In this way the inserted transgene is carried by some germ-line cells in their genome. When the offspring get this inserted foreign gene through the cells then they stably become transformed. Due to low efficiency of genomic transgene insertion, suitable marker is needed for proper and easy identification of the transformed individuals.

    - **Method of Transformation:** Insects normally have four life stages viz. egg, larva, pupa and adult, though there are so many variation as the entire insect does not possess all thesefour stages in their life; some of them may be absent. For successful transformation generally eggs are used, though adults are also in use less frequently. Most common method for inserting desired gene within the egg of the species concerned for modificationis microinjection system which comprises of a stereozoom microscope, a mechanical stage, micromanipulator and a DNA injection mechanism system (manual or electronic air-pulse system). In this process the needle is aligned along with micromanipulator. Egg micropyle is oriented towards the needle by moving the mechanical stand. DNA having the desired genetic material for transformation flanked by TIR along with transpoase enzyme is transferred to the region containing the germplasm of the early embryo. During the embryonic

development transpoase enzyme acts on the TIR and initiate transposing of the gene of interest on a chromosome. As a result the offspring born from that egg is become genetically modified. There are two ways of modification through microinjection; in one method the trans-gene is injected into the follicle of ovary prior to oviposition and in another method the foreign genetic material is injected in the haemocoel of female insect for uptake of the genetic material into egg follicles along with vitelline.

- **Classification of Transposable Elements:** There are three classes of transposable elements (Pimpinelli *et al.*, 1995)-

  - Class I elements – These element mediate transposition through reverse transcription (They become as like RNA for transposition).
  - Class II elements- These element mediate transposition transposition directly between DNA, travelling from one position to another position completely (they does not copy) (Pimpinelli *et al*., 1995). In insects for germ-line transformation generall Class II elements are used.
  - Class III elements- They are generally termed as miniature inverted-repeat transposable elements (MITES); these elements are small and incapable of encoding any protein but they are able to non-replicative relocation to a new insertion locations.

- **Different Transposable Elements**

  - **Hermes:** This element belongs to the hAT family closely related to hobo, first discovered in the housefly namely *Musca domestica* (Atkinson *et al*., 1993; Warren *et al*., 1994). Hermes and piggyBac, were mainly applied for transforming of insects other than non drosophilids, though they are extensively used and testing results indicated their applied purposes. These elements had been used for transforming several insects like Yellow fever causing mosquito, *Aedes aegypti* (Jasinskiene *et al*., 1998), Southern house mosquito, *Culex quinquefasciatus* (Allen *et al*., 2001), Mediterranean fruit fly, Ceratitis capitata (Michel *et al*., 2001), Stable fly, *Stomoxys calcitrans* (O'Brochta *et al*., 2000), Squinting bush brown butterfly, *Bicyclus anynana* (Marcus *et al*., 2004) Red flour beetle, *Tribolium castaneum* (Berghammer *et al*., 1999) etc.

  - **Mariner and Minos:** The mariner element was first recognized from *Drosophila mauritiana* (Jacobson *et al*., 1986). *mariner* was first discovered transposons used in non- drosophilids, and belongs to Tc family. This 1,290 bp long element comprises a 21-bp inverted terminal repeat. Insects transformed using this type of element are Yellow fever mosquito, *Aedes aegypti* (Coates *et al*., 1998), Housefly, *Musca domestica* (Yoshiyama *et al*., 2000) etc.

    Minos was 1st successfully used for transformation of non-Drosophilu insects by changing in germ line (Loukeris *et al*., 1995). It has 1.4-kb length and a characteristic 100 bp long inverted terminal repeats with 60-bp intron. It belongs to Tc1/mariner super-family (Franz *et al*., 1994). This element was applied in germline transformation of malaria causing mosquito of Indo-Pakistan region, *Anopheles stephensi* (Catteruccia *et al*., 2000), Silkworm moth, *Bombyx mori* (Uchino *et al*., 2007).

- **piggyBac:** Most commonly used element for insect transformation. piggyBac inserts exclusively into target sites with a sequence of TTAA (Cary *et al*., 1989). This element has 2.5 kb length with an open reading frame of 2.1-kb designed for encoding a transpoase enzyme and 13-bp inverted terminal repeat. Insects has been transformed using this element are Pink bollworm, *Pectinophora gossypiella* (Peloquin *et al*., 2000), Silkworm, *Bombyx mori* (Tamura *et al.*, 2000), Harlequin ladybird, *Harmonia axyridis* (Kuwayama *et al*., 2006), Sawfly, *Athalia rosae* (Sumitani *et al*., 2003), Housefly, *Musca domestica* (Hediger *et al*., 2001), New World screw worm fly, *Cochliomyia hominivorax* (Allen *et al*., 2004), Mediterranean fruit fly, Queensland fruit fly, *Bactrocera tryoni* (Raphael *et al*., 2010), Oriental fruit fly, *Bactrocera dorsalis* (Handler & McCombs, 2000), Mexican fruit fly, *Anastrepha ludens* (Condon *et al.*, 2007), Asian tiger mosquito, *Aedes albopictus* (Labbé *et al*., 2010), Indo-Pakistan malaria mosquito, *Anopheles stephensi* (Nolan *et al*., 2002), New World malaria causing mosquito, *Anopheles albimanus* (Perera *et al*., 2002), African malaria causing mosquito, *Anopheles gambiae* (Grossman *et al.*,2001) etc.

2. **Paratransgenesis:** The principal idea behind paratransgenesis is the reduction of disease spreading ability and competence of a vector by modifying the endosymbiont at genetic level. There is a range of possibilities for effective results which depends on how much tightly the endosymbiont is associated with its host insect. *Wolbachia* species is the popular bacteria that occupy the intracellular space of the insect cells and they are not free living. They are non-infectious in nature transmitted from one individual to another individual by vertical transmissions only that means from mother to offspring. *Wolbachia* is used in a non-GM strategy like in Incompatible Insect Technique (IIT) which can be said as a variant of SIT as described by Chambers *et al*. (2011). In IIT the principal mechanism is based on cytoplasmic incompatibility, induced due to the action of an intracellular bacterium *Wolbachia pipientis* which results in embryonic mortality (Laven, 1967; Brelsfoard *et al*., 2008; Alphey *et al*., 2010). Induction of refractoriness to dengue virus was done using *Wolbachia* in open field in Australia (Hoffman *et al*., 2011).

   Wolbachia can be found in various insects. Vector of dengue virus *Aedes aegypti* does not carry *Wolbachia* and generally does not get infection with the bacteria. *Wolbachia* are transmitted from mother to offspring, like mitochondria, but causes manipulation in the host's reproductive biology, such that they tend to spread through the species. In the presence of Wolbachia dengue virus cannot multiply inside the mosquito. *Wolbachia* also induces feminization that means transformation of genotypic male into phenotypic female. They can also modify within male sperm and also reduces egg number in host. *Wolbachia* infection causes several effects like altering the biting position, affects nutritional status of the host, reduces the lifespan of host, affect brain tissues of the host, prevent virus multiplication and indirectly kill the virus.

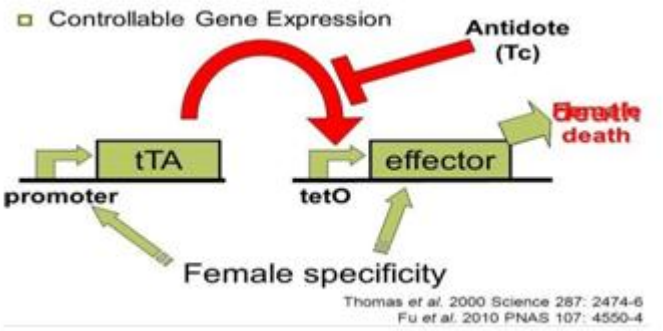
3. **Ridl (Release of Insects Carrying a Dominant Lethal):** In case of Sterile insect technique (SIT) for pest management employs liberation of sterile irradiated males into the native habitat. In this technique female elimination is required as they do not perform any function in control mechanism. For fulfilling these requirement different methodologies of sex separation including mechanical seperation from pupal mass, separation based on the adult emergence time etc are practiced with unsatisfactory

outcomes. Due these processes induced chromosomal aberrations may occur which reduces overall fitness of the transformed insect. In this context an alternative way was demonstrated in fruit fly *Drosophila melanogaster* by Thomas *et al*. (2000) and they referred it as 'Release of insects carrying a dominant lethal' (RIDL). In this approach an antibiotic tetracycline is used and the element which controls the transcription and derive the expression of the concerned antibiotic is called tetracycline repressible trans-activator fusion protein or in brief tTa. When antibiotic tetracycline is absent, any gene can be expressed by tTa that is under control of tetracycline repressive element, tRe which is designed for elimination of females.The tTA is the main driving factor of this system; this fusion protein is mainly responsible for combining the properties of Tn10 TetR protein that is the sequence-specific tetracycline-repressible DNA binding properties with the eukaryotic transcriptional activation properties of Herpes virus VP16. When the tetracycline does not present, tetO sequence get bind with this protein and transcription is initiated from the nearby minimal promoter. Here an effector gene responsible for cell lethality is also placed which is controlled by tetO. There are several number effector genes are available and they should kill the cell in case of over expression.

Requirement of promoter that is female specific is essential for making this system specific to females, where tTA protein is controlled by that promoter. As a consequence tTA protein expression occurred only in females. As a result males are capable of survival in the presence or absence of tetracycline, but tetracycline is required for the survival of female insect and in absence of tetracycline they got killed. In an alternative way Thomas *et al*., 2000, used a female specific effector gene that only lethal to females. They used a gene, *msl- 2* responsible for sex determination whose expression is only restricted to male. Ectopic expression in females resulted in over-expression of X-linked genes which was proved to be lethal.

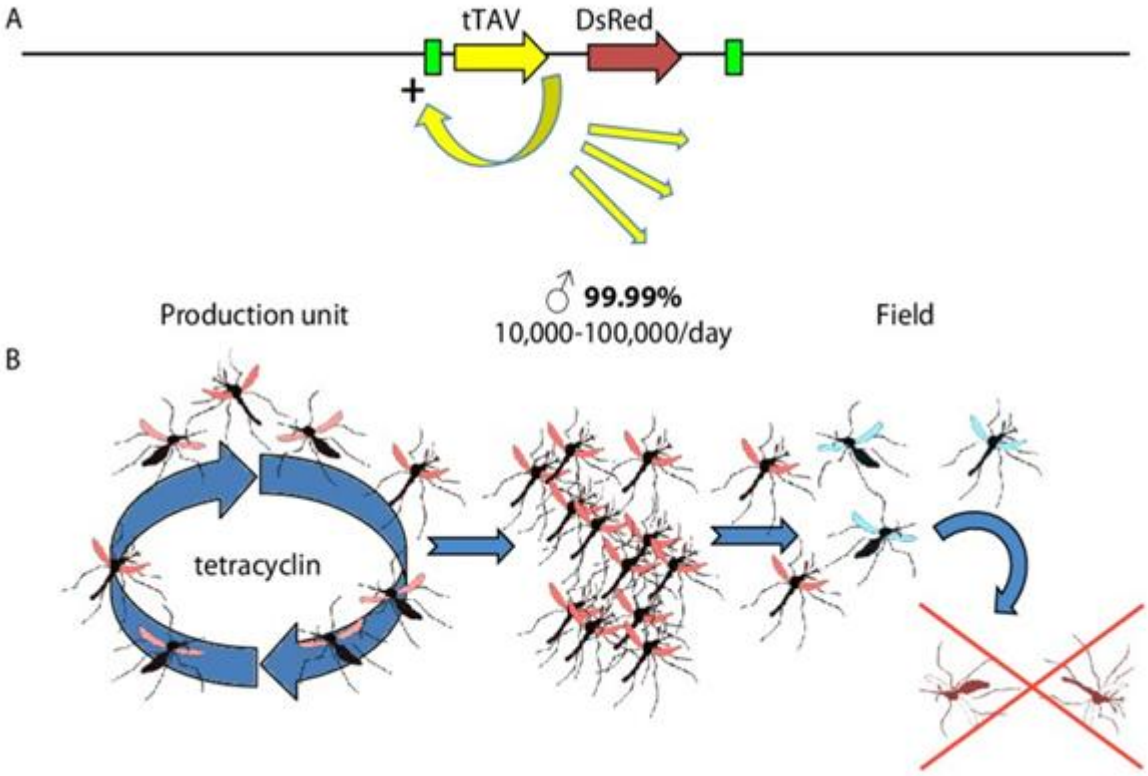
Different assessment processes have performed for assessing several RIDL/fsRIDL strains for checking their potentiality for applying in the field. Characteristics that were considered for further assessment in laboratory for checking the future performance are living duration, competitive ability for mating with other males and penetrating ability of the interested trait when reared on natural host plants (comparing with artificial diets). Survival of the modified insects on different crops and trials on mating competitiveness can be carried out in different field cages. Then greenhouse trial can be conducted for checking whether the RIDL insects will suppress the wild population. In addition to population suppression, RIDL also provides effective tool for insecticide resistance management. Therefore, it can be said that use RIDL and insecticides synergistically have a huge potential for reducing overall chemical insecticide usage as well as increasing insecticide efficacy and resistance management.

This genetic technology proved greatest potential for controlling several vector mosquitoes like *Aedes aegypti* and other agriculturally important pest like Mediterranean fruit fly, olive fruit fly, pink bollworm, *Plutella xyllostella* and *Aedes albopictus*. The cross- species function of RIDL and fsRIDL systems adopted in Tephritid fruit flies, mosquitoes and Lepidoptera, suggested that this system should be more easily transferred to other target insects in next future, offering a novel way for pest management and implementation of IPM tools widely in agricultural and human health issues.



tTA is placed under the control of a suitable promoter, e.g. constitutive, female-specific, embryo-specific, etc. when tetracycline (Tc) is absent, tTA binds with tetO , results in expression of an effector molecule leading, in the case of a lethal effector, to death. In the presence of Tc, tTA binds Tc; the Tc-bound form does not bind DNA, therefore does not activate expression of the effector, and the system is inactivated.

Source: Thomas *et al.* 2000



Source: *Jérémy Bouyer and Eric Marois,* 2018

Principle of the release of insects carrying a dominant lethal gene (RIDL). (A) scheme of the transgene. The tetracycline activator variant (tTAV) protein binds to its own promoter, activates its own transcription and perturbs overall gene expression in the cells, resulting in mosquito death, unless tetracycline that binds and inactivates tTAV is provided. (B) During mass rearing in the production unit, mosquitoes develop normally in the presence of tetracycline. For an intervention, males are sorted at the pupal stage

(based on the smaller size of male pupae). Once released, they mate with wild females whose progeny will die due to unrestricted tTAV activity.

4. **Sperm-mediated transformation:** The standard procedure for insertion of foreign desired plasmid DNA inside the genome of an organism requires microinjection of that DNA in the early stage pronucleus (mice; Palmiter & Brinster, 1986), into developmental stage having one to two cell only (zebrafish; Fadool *et al.*, 1998), or inside the egg at early embryonic stage (fruit flies; Rubin & Spradling, 1982). Previously importance was given for transforming honeybee eggs by plasmid DNA through microinjection or lipofection (Bachiller *et al.,* 1991). Though this gave satisfactory result but rearing of transformed honey bee embryos into adults was very much troublesome as the rejection rate of these manipulated embryos by worker bees was very high. The worker honeybees can detect the abnormalities; as a result of this the nursing bees destroy the manipulated embryos. Therefore several researches suggested for using of sperm for delivery of foreign DNA molecule (Zani *et al.,* 1995; Shamila & Mathavav, 1998). This method comprises of sperm collection from various drones artificially, then inoculation of plasmid DNA and after that transferring the sperm into the oviducts of a virgin queen honey bee. The sperm usually move from oviduct to spermatheca and stored for several months to years. The use of this method for genetic transformation by introduction of foreign plasmid DNA would take advantage of the in vivo colony rearing conditions for transgenic progeny, as well as not normally being physically damaging to honey bee queens. It was observed that DNA can bind to the external side of the sperm, and during fertilization the foreign can be inserted inside the egg through the sperm (Atkinson *et al.*, 1991). Successful transformation of *Bombyx mori* has been achieved by injecting the foreign DNA into the testis of insect during larval stage. (Shamila & Mathavav, 1998).

VII. APPLICATION OF GM INSECT

1. Public health issues

- **Genetically modified malaria causing mosquito:** Mosquitoes are genetically modified to produce protein which causes disruption of life cycle malarial by introduction of Gene SM 1. This gene also prevents entry of malarial parasit inside the mid gut and reaching salivary glands. Green fluorescent protein (GFP) is introdiced inside the GM mosquitoes for their identification which makes their eyes glow green under UV light. The generation of transposition system results in the formation of mosquitoes having some of the immune response genes which are chimeric mentioned above. Defensin gene was extracted from *Aedes aegypti*, and placed using the vitellogenin promoter from this species, that was inserted again into *Aedes aegypti* by transposition using the transposable element *Hermes* (Kokoza *et al.,* 2000). The mid-gut specific carboxypeptidase promoter has been used to drive expression of the SM1 synthetic polypeptide in transgenic lines of Anopheles stephensi. This synthetic small peptide binds to the midgut and salivary glands and blocks transmission of *Plasmodium berghei*, the pathogen of rodent malaria, in these transgenic lines. When the same promoter was used to drive the expression of bee venom phospholipase, which inhibits oocyst formation in the blood meal, a reduction of *P. bergei* was observed in transgenic lines of *Anopheles stephensi*. Attacking Plasmodium in the midgut, or at the midgut/ hemocoel boundary, has been a favored

site of transgeneexpression since it is in these tissues that the numbers of the parasite are at their lowest and so represent perhaps its most vulnerable stage in the mosquito.

- **Chagas disease:** *Rhodococcus rhodnii* is a pathogenic endosymbiont generally house inside the hind gut of some blood sucking kissing bug also called as triatomine bug, *Rhodnius prolixus*, that is known for transmitting the pathogen *Trypanosoma cruzi*, causal organism of sleeping sickness. The main action performed by the *R. rhodnii* is making the provision of vitamin B complex for the host insects as they cannot acquire vitamin B complex in the absence of this endosymbiont. Coprophagy is the process of acquiring *R. rhodnii* by the nymph of these bugs, in which the nymphs feed on the feces for getting the concerned endosymbiont. The bacteria is modified genetically for production of an antimicrobial peptide that is cecropin A which is known for antagonizing the pathogen *Trypanosoma cruzi* so that the vector bug cannot transmit the disease caused by *Trypanosoma cruzi*. For this purpose foreign gene encoding the antibody is introduced inside the bacteria and these modified bacteria are given in the fecal matter of the bug from which the nymphs take the transformed bacteria. Therefore the modified bacteria secret the concerned antitrypanosomal peptide or transmission-blocking antibody inside the midgut of the kissing bug and kill the pathogen *Trypanosoma cruzi*.

2. **Diamondback moth:** These moths were modified through making an assemble of a "lethality gene" named as *tetracycline transcriptional activator variant* (*tTAV*), with a combination of DNA molecule obtained from *Escherichia coli* bacteria and from the herpes simplex virus; and after that the DNA assemble was introduced inside insects. Concept behind this experiment was thought as if the engineered male moths mate with female moths under open condition in the wild, then the *tTAV* gene of the modified males will pass in the population of next generation. The gene will show its lethality towards female progeny and the females will die at larval stage. But in case of male progeny they will survive and will inherit half *tTAV*. As like a chain the grown up modified male of next generation also mate with other normal wild females but the female progeny of them also die which result in shrinking of population. The insects manufactured by Oxitec through genetic engineering possess a gene encoding a fluorescent marker for identifying them in the wild population. The modified males also have another advantage as they can be used as an effective tool for insecticide resistance management and effective use of insecticides as well asGM crops that is resistant to DBM. The reason behind this is, the males released in the field are bred from a susceptible line and modifiedunder laboratory condition so that no resistant gene is no present in them.

3. **Mediterranean fruit fly:** The Mediterranean fruit fly is a threat to global agriculture. They causes heavy damage by inflicting injury to more than 300 crops infest like wild fruit, vegetables and nuts. GM-males have been derived by transgenesis possess a gene lethal to females which arrest development of pupa meant for female fly and kill them, hence also called as "pre-pupal female lethality". After passing several generations, the diminishing of fly population occurs as because lacking of females in the population which causes the male fly fail to find the mate. For making the lethal gene inactive in the male body the lethal gene is silenced by using tetracycline antibiotic and the males are utilized for breeding under laboratory condition.

Helen Wallace from Genewatch, which is an organisation perform the monitoring of genetic technology utilization, stated that "Fruit grown using Oxitec's GM flies will be contaminated with GM maggots which are genetically programmed to die inside the fruit they are supposed to be protecting". In addition she also mentioned that when the GM flies evolve resistance as well as breed in the areas having tetracycline contamination as it is a common chemical used in agriculture, then the mechanism of lethality was tend to be failed in case of longer term.

4. **Pink boll worm**: Thomas Miller and his coworkers at the University of California, Riverside, USA, did experiment for the management of pink bollworm, *Pectinophora gossypiella* infested cotton, by modification of these moths with *Notch* mutant gene through genetic engineering and releasing the modified moths in the wild population. Under warm temperature condition normal egg development was driven by normal *Notch* gene which also prevents normal egg development under cool temperature. Mating between mutant and wild population led to the production of progenies with less fecundity. It caused failure in perpetuation in due course of time. Confined field trials have been performed for this study.

5. **Genetically Modified Trichogramma**

Gene	Source	Against
Parathion hyrdolase gene	*Pseudomonas diminuta & Flavobacterium*	Organophosphate
Acetylcholine estrase gene	*Drosophila melanogaster & Anopheles strephansi*	Organophosphate
Esterase B1 gene	*Culex* spp.	Organophosphate

6. **Application in Sericulture:** Genetically modified silkworm was modified for the production of human growth hormone and human collagen proteins. Modified silk moth possesses modified silk gland which generally does expression of the introduced L-chain gene and Green Fluorosent Protein. Cocoons generated by these transformed insects contain recombinant human collagen that can be used for surgery. Silkworm larvae are modified to assist them for producing spider milk protein for manufacturing of bullet proof vests, parachutes and artificial ligaments (Lewis, 2006).

EGT gene prevents the transgenic silkworm from molting and interruots metamorphosis from pupae to adult (Zhang, 2012). The plasmid DNA was given inside silkworm moth eggs through sperm-mediated gene transfer.

VIII. COMMERCIALLY RELEASED

- Predatory mites – In 1997 in US
- Pink bollworm – In 2001 in Mexico
- Anopheles mosquito – In 2002 in New Delhi and UP
- Screw worm fly – Exported from Libya to Kenya and Central America

IX. CONCLUSION

Insects are essential resources for maintenance of global ecological balance and they developed a wide range of variation for adapting in their respective environment. Economical as well as social issues are hampered by the interaction of the insects with the human interest as the insects are responsible for vectoring several human and animal diseases along with huge losses of crops. They are also responsible for economic and social harm worldwide through the transmission of disease to humans, animals and damage to crops. In this context genetic modification of insects can be a novel way to combat the insect pests. However, implementation of regulatory guidelines is essential for adopting this technology which is not yet developed totally. In our perception, it can be said that the benefits of adopting this technology is far more than the possible disadvantages of this technology. In these circumstances more researches are needed to find out the actual impact of this GM technology on the issues concerned with health and environment issues. There is great scope in future for popularizing and applying the concept and principles of respective GM technology for modifying the insects of agricultural and medical importance in our country that will boost the economy as well the health conditions of the citizen.

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