



# Advances in genetic approaches for sustainable insect pest management

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## Abstract

Insect pests have contributed significantly to agricultural losses as well as to the spread of vector born infectious diseases globally for the last two decades. Insecticides use is the most significant strategy among the farmers for pest management; however, this practice has been posing considerable hazards to population health and their surroundings. Additionally, extensive insecticide application has also contributed to resistance in a wide range of insect pests. Genetic pest mitigation strategy like sterile insect technique (SIT) has successfully eradicated various damaging pests globally. This technique is based on the mass release of genetically modified organisms into the ecosystem. However, sterilization by traditional methods declines the mating competitiveness and overall vitality of released sterile males. To overcome these limitations different sterility approaches i.e. release of insects carrying dominant lethal (RIDL), incompatible insect technique (IIT) utilizing *Wolbachia* and autosomal-linked X- chromosome shredders have also been proposed as a substitute to traditional SIT approach. Although these first-generation genetic SIT approaches signify considerable progress, each method has certain drawbacks. Thus, application of highly advanced SIT based approaches would be logistically beneficial that could efficiently separate sexes and at the same time sterilize males, while ensuring that their fitness will not be significantly affected. CRISPR has facilitated the establishment of innovative system called precision guided sterile insect technique (pgSIT) that allows concurrent sterilization and sexing, facilitates release of eggs and ensures emergence of sterile males. In the upcoming time, this technique could be utilized against both insect vectors responsible for transmission of human diseases and agricultural pests for reduction in prevalence of both human diseases and crop losses, respectively, thereby eliminating the necessity of dangerous synthetic chemicals and advancing insect pest management. This review briefly discusses all these SIT based approaches regarding their mechanisms, advantages, successful application against different target pests and drawbacks.

**Keywords:** Cytoplasmic incompatibility, Genetic pest management, Precision guided SIT, Release of insects carrying dominant lethal, Sterile insect technique (SIT), X-chromosome shredders

**To cite this article:** Asif, M. U., & Memon, R. M. (2025). Advances in genetic approaches for sustainable insect pest management. *Journal of Pure and Applied Agriculture*, 10(2), 12-21.

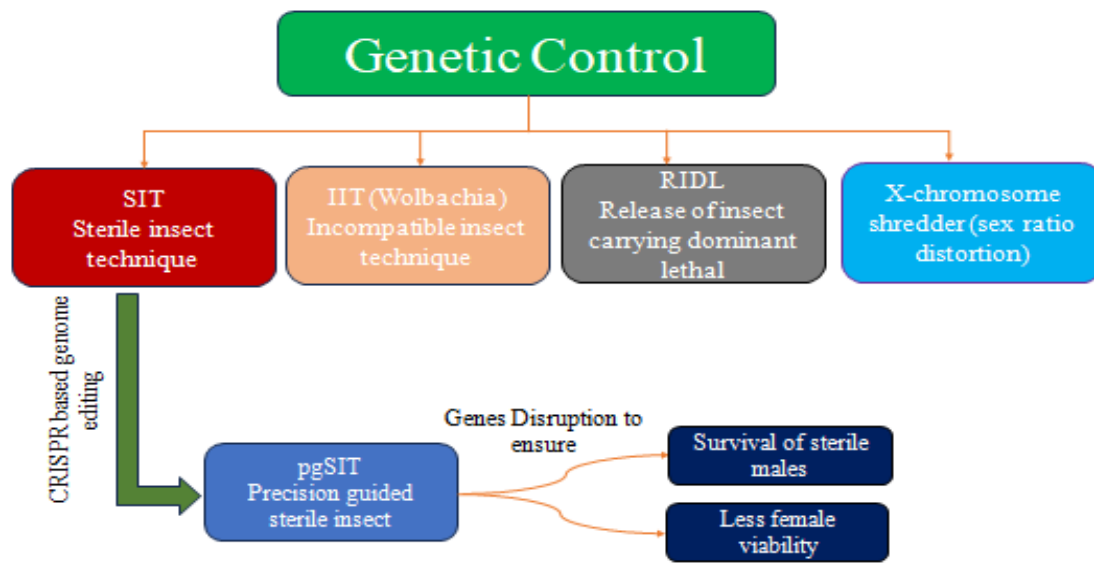
## Introduction

Global food security, climate change, vector borne diseases and environmental sustainability have emerged as significant challenges in the 21<sup>st</sup> century (Noroz et al., 2021; Omokhafa et al., 2024; Latif, & Abbas, 2025). Insect pests have contributed significantly to agricultural losses as well as to the global spreading of vector born infectious diseases for the last two decades (WHO, 2014; Deutsch et al., 2018; Ahmad & Ahmad, 2018; Rubab et al., 2020). Climate change factors and increasing temperatures are anticipated to be exacerbated by these issues (Jactel et al., 2019; Tonnang et al., 2022; Batool et al., 2025). Presently, insecticides use is among the most significant strategies for the management of insect pests; however, this practice could pose significant risks to both human health and environment (Bonner and Alavanja, 2017; Rani et al., 2021). Additionally, extensive insecticide application has led to resistance emergence among various insect pests (Liu 2015; Jurat- Fuentes et al., 2021; Ullah et al., 2023). Accordingly,

there is an exigent need for alternative pest management strategies that are economically viable, more sustainable and environmentally friendly (Qureshi et al., 2025). These strategies have become a primary focus in current pest management research.

## Genetics based techniques to control pests

Genetic pest management, also called Genetic control, is a biological approach for pests that includes the induction of required hereditary alterations through mating within species into a naturally existing population (Harvey-Samuel et al., 2017; Devos et al., 2022). Genetic control is amongst the most significant methods that relies on the extensive deployment of genetically altered organisms into the ecosystem (Fig. 1). The released organisms mate with naturally occurring insects and transfer traits that alter or reduce the abundance of target pests (Herbillon et al., 2024). The sterile insect technique (SIT) is the most frequently used approach to manage insect pests genetically (Wyss, 2000; Ying et al., 2023).



**Fig. 1** Different strategies of genetic control for insect pest management

### Sterile insect technique

The SIT was initially executed and emerged as the leading management approach for controlling the population of insect pests. It involves the mass rearing of target pests, treatment by radiation to produce sterility and then widespread releases into the natural environment for mating with their counter parts of wild type (WT) that ultimately results in no progeny (Colacci et al., 2025) (Fig. 2). This technique is extremely efficient, species-specific, and effectively integrated into large scale pest control programs to combat harmful pests globally (Klassen and Curtis 2005; Vreysen et al., 2021). The major benefit of SIT compared to other control approaches (i.e. removal of breeding places, larvicides, synthetic insecticides etc.) is that the adult males are very efficient at searching out intra-specific females and the effectiveness of technique is further enhanced as the population is suppressed. The earliest successful application of SIT against an insect was the elimination of melon fly in 1963 from Rota in the islands of Mariana (Mizuno, 2024). During the late 1970s USA and Mexico initiated the first area-wide program that inhibits the incursion of the *Ceratitidis capitata* into Mexico from Central America (Arthur et al., 2015). During 1989s and 1990s SIT was implemented to eliminate the melon fly from Okinawa and all islands of south-western Japan, permitting entry for vegetables and fruits grown in these locations to the central markets of Japan (Kuba et al., 2020). The SIT was employed to eradicate the fruit fly from north zone of Chile as a joint initiative with Peru in northern areas of Chile and southern Peru. Consequently during 1995 the whole country was declared as the fly-free zone. Since that time, USA permitted entry of fruits from Chile without any obligation of quarantine treatment, resulting in significant gain to the economy of Chile (Enkerlin, 2021). Argentina has also

executed significant SIT programs against fruit fly in various fruit growing provinces, achieving remarkable success in complete eradication from some areas. Similarly, Mexico has also initiated the SIT program to eradicate various species of *Anastrepha* from northern side of Mexico (Enkerlin, 2021). An unprecedented success was gained in insect pest management by utilizing SIT to eradicate screw worm (*Cochliomyia hominivorax*) a harmful fly, detrimental to human health, wildlife and livestock, from Central and North America (Klassen & Curtis 2005; Scott et al., 2017).

Tabashnik et al. (2021) documented the successful elimination of pink bollworm recognized as one of the most destructive agricultural pests globally, from the cotton producing regions of the USA and northern Mexico. A successful outcome was attained through a coordinated and multitactical program, occurring a century after the pest has initially invaded both nations. The program involved the aerial releases of billions of sterile pink bollworm moths, along with the cultivation of transgenic cotton that produces insecticidal proteins from *Bacillus thuringiensis*. This achievement has resulted in economic, social and environmental benefits. Computer simulation analysis and field data of 21 years from Arizona suggested that these two strategies synergistically interacted to effectively reduce the pest population. The elimination of pink bollworm significantly mitigated the damage inflicted on cotton crop, along with reducing the reliance on insecticide (Table 1). These conventional approaches have depended upon traits that damage DNA for sterilization, considerably declining the mating competitiveness and on the whole fitness of released sterile males. To overcome these limitations, several sterility-based strategies have been proposed, including the microbe-mediated incompatible insect technique (IIT) using Wolbachia (Sinkins, 2004; Panagiotis & Bourtzis, 2007), genetically engineered approaches like release of insects carrying a dominant lethal gene (RIDL) (Thomas et

al., 2000), female-specific RIDL (fsRIDL) (Fu et al., 2010), and autosomal-linked X-chromosome shredders (Windbichler et al., 2008).

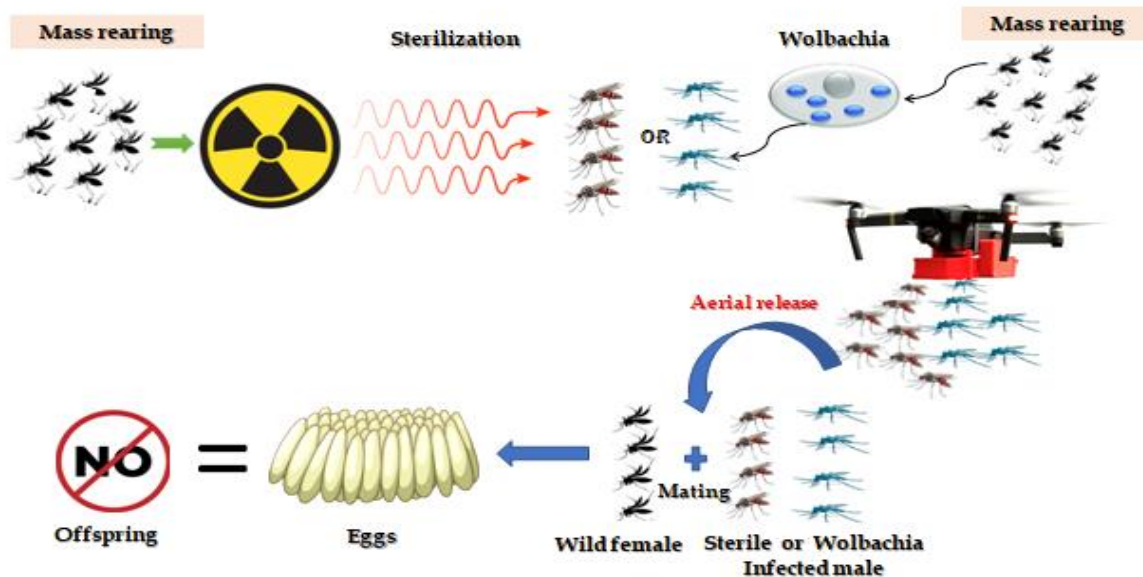


Fig. 2 Population reduction using SIT or Wolbachia mediated IIT

### Incompatible insect technique (IIT)

During the last few years, the IIT has gathered substantial interest as a substitute to traditional sterility approach (Hughes et al., 2014; Ritchie et al., 2018). It depends upon males infected with *Wolbachia* that are unable to produce fertile progeny once coupled with their wild counterparts (Matsufuji & Seirin-Lee, 2023). *Wolbachia* is a bacterium that is symbiotic intracellularly transmitting infection into extensive variety of organisms belonging to phylum Arthropoda including mosquitoes, and almost 40% of arthropods in environment are thought to be infected by it. As it replicates within the cell of host, it could not enter the sperm and is mostly located in the female reproductive organs; consequently, it spreads to progeny from female through ovum (Werren et al., 2008). It leads to the initiation of reproductive mechanism called cytoplasmic incompatibility (CI) in host insects. In this process uninfected female reproduction is hindered due to mating with infected male through abnormal cell multiplication in the initial stages of egg, leading to hatching failure (Werren et al., 2008) (Fig. 2). Few decades back, *Wolbachia*-induced CI was utilized as a strategy for the first time to reduce the natural abundance of *Culex pipiens*. Recently there have been substantial achievements towards the application of IIT for the suppression of mosquito vectors. Moreover, *Wolbachia* based self-supporting techniques also exist that target the replacement of population via CI-induction and pathogen inhibiting strains; though the usefulness, sustainability and effectiveness of this approach require future research (McGraw and Neill 2013; Hoffmann et al.,

2014). *Culex pipiens* lines infected with *Wolbachia* were chosen and evaluated in controlled conditions for expression of cytoplasmic incompatibility and suppression of four populations representing four islands of south-western Indian Ocean i.e. LaReunion, Mauritius, Grand Glorieuse and Mayotte (Atyame et al., 2011, 2015). The outcomes of these controlled cage trials were very encouraging, demonstrated that *Wolbachia* infected males of *C. pipiens* were completely incompatible (100 % cytoplasmic incompatibility) with their counterpart females collected from same four locations (Atyame et al., 2011). Lately, cytoplasmic incompatibility based IIT approach was evaluated experimentally in China (Zheng et al., 2019), USA (Crawford et al., 2020) and Australia (Beebe et al., 2021). In the former two experiments, a combined approach of SIT and IIT was evaluated to indemnify the issue of contamination of female mosquitoes. The results of both these experiments indicated more than 80 % efficiency in suppression of wild females. The latter experimental work investigated the IIT utilizing multidirectional approach and proved highly successful for suppression of mosquitoes. Even though these investigations confirmed the success of the IIT based on the combined approach and in confined areas for minimum duration, the findings indicated that the IIT might be a realistic approach for managing population abundance of mosquitoes. Consequently, investigating the long-term global efficacy of the IIT method should be considered as a critical next step, as sustained release strategies that encompass extensive areas are expensive and necessitating a thorough evaluation of alternative control strategies prior to their application (Matsufuji & Seirin-Lee, 2023).

**Table 1** Successful applications of SIT for eradication of insect pests

Target Pest	Location	Year	Methodology	Outcome	References
Screw worm ( <i>Cochliomyia hominivorax</i> )	United States: Texas and Westers States	1962-66	Release 200- 1000 sterile males per km <sup>2</sup> per week	Declared eradication in Texas and Mexico in 1964 and in USA in 1966	(Wyss, 2000)
Mediterranean Fruit fly ( <i>Ceratitidis capitata</i> )	Mexico and Guetmala	1978	Produced 500 million and 3500 million sterile flies per week in Mexico and Guaetmala,	Eradication in 1982	(Villasenor et al., 2000)
Screw worm ( <i>Cochliomyia hominivorax</i> )	Central America	1984-2001	Sterile male releases	Declared complete eradication	(Klassen & Curtis, 2005)
Fruit fly ( <i>Bactocera cucurbitae</i> )	Okinawa	1986-1990	Sterile male releases	100 % eradication of melon fly	(Kuba et al., 2020)
Mediterranean Fruit fly ( <i>Ceratitidis capitata</i> )	California and Florida, USA	1994	Sterile male releases	Eradicated successfully in 1996.	(Dowell et al., 2000; Barry et al., 2004)
Codling Moth ( <i>Cydia pomonella</i> )	Canada; British Columbia	1994	Released irradiated codling moths	Population Suppressed	(Bloem & Bloem, 2000)
Tsetse ( <i>Glossina austeni</i> )	Unguja Island, Zanzibar	1994-97	8.5 million sterile males were released	Population completely eradicated in 1997	(Vreysen et al., 2000)
Fruit fly ( <i>Ceratitidis capitata</i> )	Spain	2003-2006	IPM program based on SIT	90% reduction of target wild population	(Pla et al., 2021)
Sweet potato weevil ( <i>Cylas formicarius</i> )	Okinawa, Japan	1999-2012	460 million releases of sterile weevils	Complete eradication. No wild weevils were caught in traps since 2009.	(Himuro et al., 2022)
Mosquito ( <i>Aedes aegypti</i> )	Cuba	2018	1,270,000 irradiated individuals were distributed in the 50-ha target area	Population completed eradicated from trial area	(Gato et al., 2021)
Codling Moth ( <i>Cydia pomonella</i> )	Newzealand	2014-2019	Sterile male releases after combined use of mating disruption and insecticides	90-99% reduction in population	(Horner et al., 2020)
Pink Bollworm ( <i>Pectinophora gossypiella</i> )	Arizona USA	2006-2013	Releases of sterile male in combination with transgenic cotton	Complete Eradication in 2013	(Tabashnik et al., 2021)
Spotted Wing Drosophilla ( <i>Drosophila Suzukii</i> )	Kent, UK	2021	Sterile male releases	91 % suppression of target pests compared to untreated control sites	(Homem et al., 2022)
Mosquito ( <i>Aedes aegypti</i> )	La Reunion and Spain	2021	Sterile male releases	More than 80% reduction	(Bouyer et al., 2025)
Mosquito ( <i>Aedes albopictus</i> )	Gampaha, Srilanka	2020-2022	3,300,000 Sterile male releases	98 % suppression of adult vector mosquitoes	(Hapugoda et al., 2025)
Mosquito ( <i>Aedes aegypti</i> )	Florida, USA	2020-2022	24.1 million sterile male releases	Population reduction of up to 79% in wild adults and a 59 % in egg densities	(Morreale et al., 2025)
Cabbage Maggot ( <i>Delia radicum</i> )	Canada	2019-2022	Large scale field trials	Significant reduction in <i>D. radicum</i> in radish and daikon crops	(Fortier et al., 2025)

### Release of insect carrying dominant lethal (RIDL)

RIDL technology presents a viable alternative to numerous limitations associated with conventional SIT that have hindered its implementation while preserving its sustainability in the environment and target specific characteristics. It varies from traditional SIT in that the released individuals are homozygous for a dominant lethal gene instead of passing through irradiation to produce sterility (Alphey, 2007). The progeny as a result from mating with native populations is heterozygous for a lethal gene which causes the mortality of offspring and hence ultimate population reduction occurred because of a lessening in its ability of reproduction (Heinrich and Scott, 2000; Thomas et al., 2000). The RIDL strain is produced through the application of modern molecular biology methods. This process involves the insertion of the RIDL gene system into the insect's genome via transformation. Homozygous RIDL strain will be developed from insects having inclusion of single transgene. This undergoes through evaluation to identify a strain having a desirable trait. This testing involves assessing traits such as late-acting or female-specific lethality, as well as conducting bionomic and fitness studies. Upon the establishment of an appropriate RIDL strain, it can be produced on a large scale with a suppressor, which is a dietary supplement that inhibits the RIDL system. These insects possess a strong lethal allele that can be suppressed by administering

tetracycline or any other appropriate analogs, like chlortetracycline, while they are in the larval feeding stage. Females are eliminated, either through physical removal or a system designed to be fatal to them. For mating with wild females, their counterparts can subsequently be employed to contend with their wild types; offspring resulted from a mating between RIDL strain and natural females will not survive as the necessary suppressor is absent (Fig. 3). Releasing a significant quantity over a prolonged period will reduce, or potentially eradicate, the specific population being targeted (Wilke et al., 2009). RIDL technique that is repressible and highly efficient was initially validated in *Drosophila* models. Later, this technology was successfully implemented in the Mediterranean fruit fly, utilizing the tetracycline-repressible transactivator (tTA) to regulate the appearance of a deleterious protein (Thomas et al., 2000; Gong et al., 2005). Morrison et al. (2012) successfully created transformed strains of the pink bollworm utilizing diverse DNA constructs. These strains exhibited moderate levels to complete engineered mortality. This effect was significantly diminished in favorable conditions. Field cage studies on cotton demonstrated that field conditions significantly enhanced the mortality rate. One strain, designated OX3402C, exhibited highly penetrant and highly repressible lethality. The larval stage of this strain caused nominal damage prior to mortality when checked on host plants. The findings underscore the possible significance of an insecticide-free method for controlling pink bollworm and suggest its applicability for the management of other lepidopteran pests.

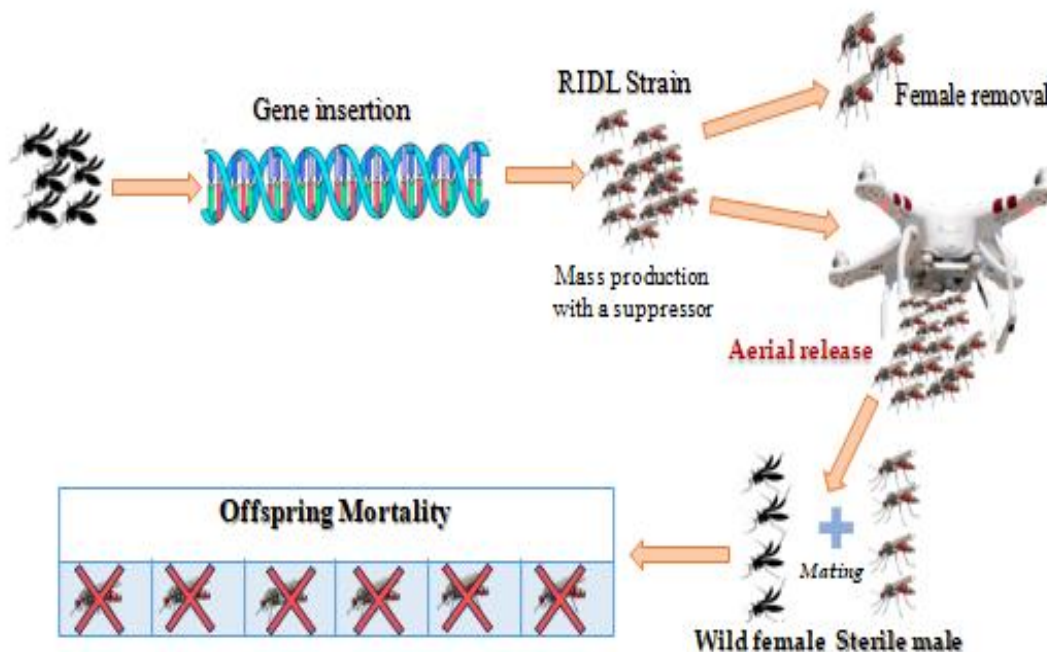


Fig. 3 Population reduction using RIDL technique



### X- chromosome shredders

Altering the reproductive sex ratio of a population significantly towards males has long been acknowledged as a potentially effective strategy for genetic control (Papathanos et al., 2014). The naturally occurring traits of sex distortion have consistently captured the attention of evolutionary biologists for long period (Jaenike, 2001). However, the synthetic biology and introduction of more efficient tools for genome-editing now permits the creation of artificial sex disruption traits aimed to understand this unexploited prospective. X- Chromosome shredding based sex-ratio disruption is more effective than SIT for suppression of population. The other approach for suppression includes biasing of the offspring sex via the interruption of each X or Y-bearing sperm, expanding the relative quantity of males or females, respectively. This type of sex-disruption approach, called X-shredding, has demonstrated significant efficacy against insects (Simoni et al., 2020, Meccariello et al., 2021). Recently, this method has evolved in *Anopheles gambiae* (Galizi et al., 2014), *Ceratitis capitata* (Meccariello et al., 2021) and *Drosophila melanogaster* (Fasulo et al., 2020). This approach has proved more effective both in experiments and theoretically than conventional SIT, regarding the quantity of insects that are required for releases (Schliekelman et al., 2005; Galizi et al., 2014; Burt and Deredec, 2018). Autosomal X-shredder constructs function as self-restraining in their fundamental form, and their releases could feasibly lead to localized and restricted suppression, if adequate number of males released over an extended timeframe (Alcalay et al., 2021).

### Drawbacks of sit technologies

Although these first-generation genetic SIT approaches signify considerable progress, each method has certain drawbacks. In these techniques, females could mate with the males co-released with them; therefore, they are regarded as ineffective agents for control which in turn hinder sterilized males to couple with wild females in the environment (Franz et al., 2021). Furthermore, a substantial population of insects is required for SIT release to guarantee that the majority of mating pairs include one released individual (Schliekelman et al., 2005). It is well-documented that insect fitness lessens with radiation, leading to a reduced competitive ability in irradiated ones in comparison to wild-type ones (Dias et al., 2021). This is specifically factual for organisms belonging to order Lepidoptera as they possess high resistance against ionizing radiation and hence need high dose of X or gamma radiation to achieve whole unproductiveness (Marec and Vreysen 2019). Moreover, authentic sex separating technique is highly desirable to remove females completely before releases, as in most cases they are involved in the dissemination of pathogens (i.e. mosquitoes). The other alternative approaches for insects control i.e. RIDL (Thomas et al., 2000), IIT based on *Wolbachia* (Xi et al., 2005; Bourtzis et al., 2014), also need accurate sex

separation technique to evade chances of releases of females. Importantly, IIT approach is relied upon systematic distribution of males infected with *Wolbachia* that are non-compatible with wild females lacking that precise *Wolbachia* strain. The unintentional release of minute quantity of females that are infected with *Wolbachia* could lead to broad range dissemination of *Wolbachia*. This results in population immunization against the specific IIT program, emphasizing the significance of efficient sorting of sexes. But with the exceptions of few specific species (Meza et al., 2018; Crawford et al., 2020), sorting insects with respect to sex could be laborious, flawed, labor demanding, laborious and species specific (Papathanos et al., 2009; Lutrat et al., 2019; Kandul et al., 2020). Thus, application of highly advanced SIT based approaches would be logistically beneficial that could efficiently separate sexes and at the same time sterilize males, while ensuring that their fitness will not be significantly affected.

### Precision guided sterile insect technique

CRISPR has facilitated the establishment of innovative system called precision guided sterile insect technique (pgSIT) to permit the release of insects at egg stage ensuring all off springs surviving to adult stage are sterile males. This is achieved by means of modern molecular genetics to remove females and at the same time sterilize males. pgSIT depends mechanistically on a genetic approach that allow concurrent sterilization and sexing, facilitates release of eggs and ensures emergence of sterile males. Notably, the release of insect at egg stage will diminish requirement of manual sex separation and male sterilization, therefore enhance scalability and decrease overall effort (Kandul et al., 2019). pgSIT employs a dual technique to disrupt genes crucial for viability of females and fertility of males leading to the essential survival of sterile males that can be implemented at any phase of life to reduce and eradicate populations (Li et al., 2021).

### Advantages of pgSIT

pgSIT does not necessitate the utilization of radiation, antibiotics or *Wolbachia* and has no long-term persistence in the environment compared to other approaches. As a whole, self-restraining property of pgSIT provides a harmless substitute to approaches that can spread and persist in the ecosystem i.e. gene drives (Champer et al., 2016). It may provide secure, scalable, effective, environment friendly substitute for the control of population abundance of wild mosquitoes leading to broad range avoidance of disease transmission in humans (Li et al., 2021). pgSIT is tremendously vigorous at genetic sexing and all together sterilizing the offsprings reproducibly with complete efficiency. Furthermore, pgSIT males are capable and could effectively strive for mating. Overall, this approach gives far greater destruction of population compared to other existing techniques, thus transforming control of wild insect populations through SIT mediated approaches (Kandul et al., 2019).

## Successful demonstration of pgSIT

Kandul et al. (2019) thoroughly develop various schemes of pgSIT in *Drosophila* that constantly provide 100% sterile adult males with full competitive and fit properties. Li et al. (2021) displayed significant production of flightless, short-lived females and fit sterile males. Notably, when released into controlled populations the pgSIT males competed with WT females thus reducing and even eradicating populations via release quantities that are attainable in field (Zheng et al., 2019; Crawford et al., 2020; Carvalho et al., 2015). Numerical models also proposed that population eradication could be achieved in field through systematic releases of 100-200 or above pgSIT eggs per wild adult of *Aedes aegypti*. Apte et al. (2024) cross separated engineered strains of Cas9 and gRNA to target and disrupt genes critical for male fertility and essential for female survival through binary CRISPR approach. This methodology resulted in over 99.5% sterility in males and over 99.9% lethality in females among the hybrid offspring. Their findings indicated that genetically sterilized males exhibit considerable lifespan, effectively achieve prolonged suppression of population in controlled cages and are anticipated to eradicate wild Anopheles populations using numerical descriptions, creating them as optimal contenders for potential distribution.

## Conclusion

With the mounting concerns regarding world-wide hunger prevention and health of public, it is possible to predict that genetic pest management will continue to be a developing area for future research that combat devastating crop pests and human diseases vectors. Thereby reducing dependence upon unsafe synthetic insecticides and transforming management of insect pests.

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