STERILE INSECT TECHNIQUE: AN ECO-FRIENDLY INSECT PEST CONTROL STRATEGY BASED ON IONIZING RADIATION

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Abstract:
Insect pests are the major biotic factors influencing the sustainable agricultural production. Some of the insect pests are harmful and detrimental to agricultural productivity, parasitizing farm animals and posing a health risk to mankind. Despite widespread use of pesticides, it is estimated that insect pests hinder 25-35% of global food production. As people become more aware of the negative impacts of pesticides, environmental friendly approaches to pest control are essential. Sterile insect technique (SIT) is one of the key control strategy currently being used for various insect pest management. Development of SIT for target insect pest requires large scale mass rearing, sterilisation using ionizing radiation and periodic release in the field. The sterile males copulate with wild, fertile females resulting no offspring and further leads to suppression and/or eradication of pest population. This technique is being employed for several insect pest including tephritid fruit fly species and vectors. BARC has developed SIT for the control of insect pests such as red palm weevil, potato tuber moth and fruit flies. Recently developed SIT module for fruit fly control is being tested under pilot field conditions in Palghar district of Maharashtra. Distribution and population dynamics of fruit fly species throughout the year was profiled; mass rearing protocols and sterility doses have been optimised. Moreover, the bacterial endosymbionts such as gut bacteria and *Wolbachia* associated with fruit fly species has been assessed. Therefore, suppressing or reducing the pest population in the field could be possible by integrating SIT and other control measures in AW-IPM programmes.

Keywords: Insect pest, radiation, SIT, IPM, bacterial endosymbionts, *Wolbachia*

1. Introduction

Agriculture in India is mainstay in economy and also majority population is dependent on farms and farm related activities. Numerous biotic and abiotic variables continue to limit agricultural production. For example, weeds, diseases and insect pests have a significant influence on agricultural output. The damage due to pests and diseases are the main concern in Indian agriculture. Green revolution has increased the yield and facilitated India to become self-sufficient, however it has also increased the use of chemical pesticides. Moreover, the green revolution technologies are saturating and thus alternative and safer technologies are required for sustainable agriculture. Various alternatives have been suggested to improve the yield and sustain the agricultural production without further damaging the environment. Managing insect pests is difficult and currently involves a variety of control methods such as gathering and eliminating affected plant parts, employing protein bait or pheromone traps for suppression, biocontrol agents and utilising specific pesticides. Nevertheless, a number of environmental conditions influence on the efficacy and efficiency of current insect pest management methods.

The sterile insect technique (SIT) is an eco-friendly and autocidal insect pest control strategy in an area-wide integrated pest management (AW-IPM) programme. During 1950s, American entomologist Dr. Edward F. Knipling and his group explored the finding made by H. J. Muller that ionising radiation causes dominant lethal mutations (DLM) in fruit fly. Then, SIT was implemented to eradicate New World screwworm (*Cochliomyia hominivorax* (Coquerel)) in Mexico, USA and Central America[20]. Later, this technique has been implemented against several insect pest and vectors management as prevention, suppression, containment and eradication[16]. Basically, SIT for target insect pest requires large scale mass rearing, sterilisation using ionizing radiation and periodic release of sterile males into the field. The sterile males will copulate with wild fertile females which leads to no offspring and further results in suppression/eradication of the pest population[21]. SIT may be applied over a large area or in a localized and focused area. At present, sterile insect release programme mostly utilise X-rays, high-energy electrons and gamma rays[4]. Use of radiation type depends upon relative biological effectiveness (RBE), penetrability, safety etc. The energy of photons (gamma rays or X-rays) must be <5 MeV (million electron volts) and below 10 MeV of electrons (IAEA, 2002). The radioisotopes source like Cobalt-60 (^{60}Co) (1.17 and 1.33 MeV) and Cesium-137 (^{137}Cs) (0.66 MeV) are currently used as gamma radiation sources. Furthermore, X-rays with an energy of <5 MeV and electrons produced by accelerators with an energy of <10 MeV are also suitable for sterilising insects[4]. At present, irradiation of more than 360 economically significant insect pests and vectors has already been conducted for basic research, pest management applications and phytosanitary/disinfestation purposes (IDIDAS database). These methods have been explored to manage the insect populations and SIT could be better alternative as it is species-specific and environmental-friendly pest management solution.

1.1 Sterile insect technique (SIT) :

1.1.1 Response of radiation on insects:

Insects are known to be more radio-resistant as compared to higher vertebrates. This is due to Dyar's Rule, which states that majority of cell divisions in arthropods only occur during the moulting process and their growth is discontinuous. The insect orders differ greatly in their radiation sensitivity, for example lepidopterans requires 130 to 400 Gy, some species from coleopterans sterilized at 40 to 200 Gy, 30 to 280 Gy in Acari, 10 to 180 Gy in hemipterans, 20 to 160 Gy in dipterans, 20 to 150 Gy in Araneae, 5 to 140 Gy in dictyopterans, 100 Gy in thysanopterans, while orthopteran insects are sterilized at doses below 5 Gy[4]. Tephritid's fruit flies have been the most studied group of arthropods with regard to sterilization by using ionizing radiation. The sterility doses of gamma radiation for different species of fruit fly ranging from 40-160 Gy[18]. While, the sterilization dose may differ by 40-60 Gy within the genera like *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus* and *Rhagoletis,* which may be due to several internal or external factors.

Lepidopterans are comparatively more radiation resistant than other orders, require a radiation dose of 400 Gy to induce sterility[22,4]. The competitiveness of sterile moths is greatly decreased by full sterilisation of lepidopteran insects. Proverbs (1962) proposed the use of sub-sterilising doses in codling moth (*Cydia pomonella* (L.)) as it showed better competitiveness as well as transmit sterility to next generation leading to the F1 progeny (Inherited sterility (IS)) that is more sterile as compared to parents. Later on, IS has been explored to several lepidopteran insect pest management[22]. The holokinetic structure of the chromosomes of lepidopteran insects plays a major role in radio resistance (Marec and Vreysen, 2019). The formation of anomalies, a decrease in adult longevity, flight and dispersal capacity, propensity to mate, nutrition and even insect mortality are examples of how radiation affects somatic cells. Therefore, insect radio-sensitivity/resistance influenced by various biological (developmental stage, age, sex, nutritional stage, size and weight, diapauses and genetic difference) and physical factors (temperature, atmosphere, dose rate, fractionation and also radiation type)[4].

1.1.2 Principles and requirements for SIT:

A large number of sterile males are released into the field condition. These sterile males copulate with wild fertile females which results no offsprings (Knipling, 1955). However, success of SIT is based on the several factors such as initial knowledge of ecology and biology of target pest, efficient mass-rearing techniques and quality control, sex segregation, effective sterilisation methods, releasing techniques, efficacy evaluation, enhancing maturation, reduce sterile male attraction to pheromone traps and also improvements through modern techniques and symbionts. The distribution and population dynamics of insect pests are thought to be important factors influencing the viability of SIT application.

According to Dr. Knipling's model, target pest population could be eradicated within five generations after introduction of SIT[21]. This suggests continuous releasing of high numbers of sterile:fertile ratio in a wild population could reduce the reproduction in population (Fig. 1). Thus, the production of healthy insects in required number and cost-effective manner is one of the principal requirements for the successful application of this technique. Presently, various artificial diets have been developed for >1300 insects to mass produce them in the laboratory for the SIT activity[18]. Additionally, the identification and examination of genetic variation in target populations, both in the wild and in laboratory environments can be crucial in determining the origins of outbreaks and reintroductions as well as in calculating rates of gene flow and dispersal among groups[4]. Several pathogenic bacteria, viruses, fungi, spiroplasma/mycoplasma and microsporidia are known to infect insects during mass rearing which hinders effective colony management. Thus, management of insect pathogens and insect colony in mass-rearing facility are crucial for successful implementation of SIT. In addition, determination of insect age/stage and radiation dose to induce sterility in target insects, assessing the reproductive parameters to ensure sterility induction, marking, releasing of sterile male ratios and techniques are the basic requirement of implementation of SIT in the field conditions.

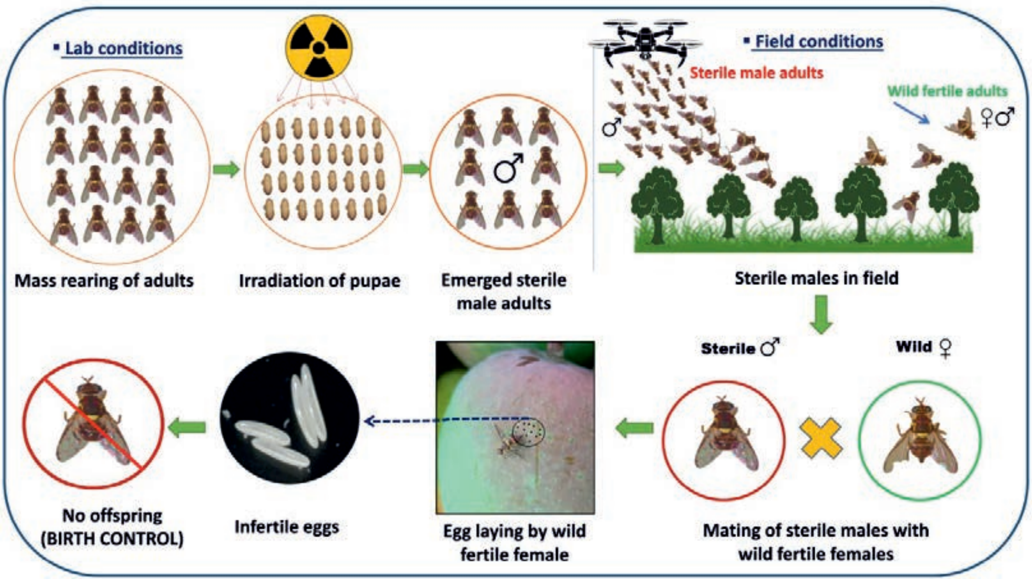

Figure 1. Sterile insect technique (SIT) strategy for area-wide insect pest management programme includes mass rearing, sterilization, releasing of sterile males in field and sterile males mate with wild fertile females which results no offsprings.

1.2 SIT and bacterial endosymbionts in insect pest management:

The SIT can be deployed as part of AW-IPM programmes for insect pest and vectors control. The peaceful applications of nuclear technology have been proved by the successful employment of AW-IPM combining SIT with other control approaches. Over the past 50 years, the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations are involved in the development and use of these eco-friendly benign technologies. After success of New World screwworm eradication program during 1954, several SIT programmes have been employed against number of insect pest species such as tephritid fruit fly species (Mediterranean fruit fly, Mexican fruit fly, Oriental fruit fly, Melon fly), codling moth (*Cydia pomonella* (L.)), pink bollworm

(*Pectinophora gossypiella* (Saunders)), cactus moth (*Cactoblastis cactorum* (Berg)), painted apple moth (*Teia anartoides* (Walker)), false codling moth (*Thaumatotibia leucotreta* (Meyrick)), some *Glossina* and mosquitoes species[20,22]. In addition, SIT is being tested and employed for the management of several insect pests (Table 1).

BARC is actively engaged in development of SIT for the management of important insect pests in India. The major insect pests like red palm weevil (RPW) (*Rhynchophorus ferrugineus* (Olivier)), potato tuber moth (PTM) (*Phthorimaea operculella* (Zeller)) and tephritid fruit fly species have been selected for SIT development[11,27]. Red palm weevil is a dominant insect pest of coconut and other palms species worldwide. Sterility doses and suitable mass rearing techniques have been standardized[25]. Under coordinated BRNS projects, field trials were conducted in collaboration with agricultural universities (Maharashtra, Karnataka and Kerala) to assess the viability of SIT. The wild RPW population was significantly suppressed and the number of infested palms decreased as a result of the introduction of sterile RPW males in coconut growing hotspots areas. Potato tuber moth is a key insect pest of potato crop both in field and during storage. Studies were conducted in association with Indian Council of Agricultural Research (ICAR) institutes to assess the usefulness of SIT for the control of PTM under storage conditions. The PTM population was greatly suppressed after releasing of PTM sterile males.

Table 1. Examples of 'AW-IPM programmes integrating with SIT' for insect pest control and type of control strategies (Source: Hendrichs et al., 2021).

Insect pest (Scientific names)	Country/ies	Control strategies applied
Diamond back moth (*Plutella xylostella* (Linnaeus))	Mauritius	Suppression
Oriental fruit fly (*Bactrocera dorsalis* (Hendel))	Thailand, Vietnam	
Onion maggot (*Delia antiqua* (Meigen))	Netherlands	
Sweet potato whitefly (*Bemisia tabaci* (Gennadius))	Europe, USA	
Serpentine leaf miner (*Liriomyza trifolii* (Burgess))	Europe, USA	
False codling moth (*Thaumatotibia leucotreta* (Meyrick))	South Africa	
Codling moth (*Cydia pomonella* (L.))	British Columbia, Canada	
West Indian fruit fly (*Anastrepha obliqua* (Macquart))	North west Mexico	
Tsetse fly (*Glossina austeni* (Newstead))	Unguja Island, Tanzania	

West Indian Sweet potato weevil (*Euscepes postfasciatus* (Fairmaire))	Japan	Eradication*
Sweet potato weevil (*Cylas formicarius* (F.))		
Old worm Screwworm (*Chrysomya bezziana* (Villeneuve))	Australia	Eradication^
Codling Moth (*Cydia pomonella* (L.))	Brazil	
Cactus moth (*Cactoblastis cactorum* (Berg))	Mexico	
Painted Apple moth (*Teia anartoides* Walker)	New Zealand	
Mexican fruit fly (*Anastrepha ludens* (Loew))	North-east Mexico Brazil, Croatia, Israel, South Africa, Spain	Suppression
	Mexico	Containment
	North-west Mexico	Eradication*
	Baja California	Prevention
New world screwworm (*Cochliomyia hominivorax* (Coquerel))	North and C. America	Eradication*
	Panama	Containment
	Libya and Florida keys	Eradication^
Pink bollworm (*Pectinophora gossypiella* (Saunders))	USA, northern Mexico	Eradication*
	California, USA	Containment
Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann))	Argentina, Mexico, Southern Peru	Eradication*
	Guatemala-Mexico, Peru-Chile	Containment
	Los Angeles Basin in CA, USA, Southern Australia	Prevention, Eradication^
Melon fly (*Zeugodacus cucurbitae* (Coquillett))	Japan	Eradication* & prevention
Queensland fruit fly (*Bactrocera tryoni* (Froggatt))	South eastern Australia, western Australia	Containment Eradication^

Eradication;* Eradication (In infested area); ^Eradication (Introduction in pest free area), Prevention –in pest free area

Fruit fly species belongs to family Tephritidae are the major insect pests on economically important fruits and vegetables in India and the world. It is well known that fruit flies have a greater invasion behaviour, increased adaptation and reproduction; hence important candidates for AW-IPM programme to curtail economic losses. The distribution and population structure of fruit fly species in India has been profiled (Hadapad et al., 2017) (Fig. 2).

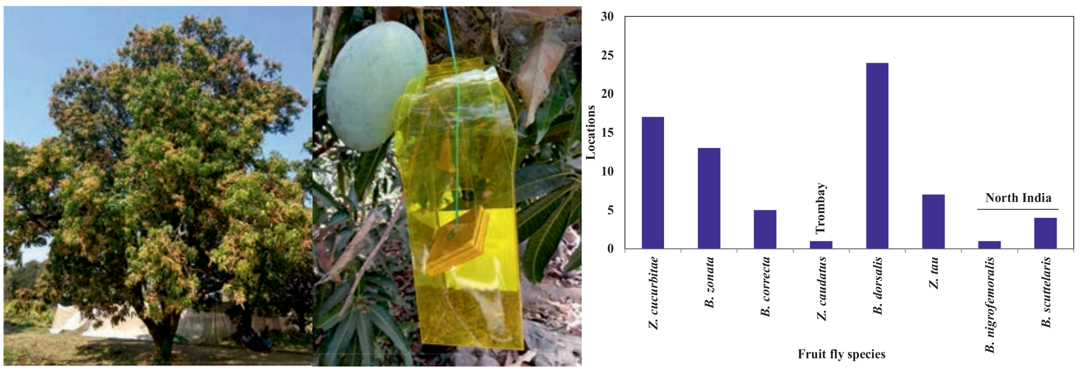

Figure 2. Distribution and population structure of fruit fly species in India.

Among them, Oriental fruit fly (*B. dorsalis*), Peach fruit fly (*B. zonata*), Guava fruit fly (*B. correcta*), Melon fly (*Z. cucurbitae*) and *Z. tau* are the dominant species distributed in India (Fig. 3). Moreover, we have studied the population dynamics of fruit fly species in mango and other fruits orchards in and around Dahanu region of Palghar district of Maharashtra. The Oriental fruit fly, Peach fruit fly and Guava fruit fly are the major fruit fly species distributed in this region causing significant yield loss and reduced market value. To assess the feasibility and to demonstrate the SIT; we have optimised the mass rearing protocols based on natural hosts, artificial diets and sterility doses. In collaboration with ICAR institutes and agricultural universities of India, this technology is being tested in pilot fields[28,11,19]. Moreover, the tomato leaf miner (*Tuta absoluta* (Meyrick)) is an invasive insect pest of tomato and other solanaceous crops. The distribution and the genetic diversity were studied with the help of mitochondrial cytochrome c oxidase subunit I (COI) gene analysis in relation to SIT[12]. The feasibility and integration of SIT with other biocontrol agents for this insect is evaluated[10,23].

Insects harbour numerous bacterial endosymbionts which are vital to their biology and fitness of the insects. Certain bacterial endosymbionts associated with insects are used to improve the fitness of sterile insects and are being explored in insect pest and vector management[7]. Among them, the reproductive parasite *Wolbachia* has ability to alter the reproductive functions and causes parthenogenesis, male killing, feminization and cytoplasmic incompatibility (CI) associated with insects including fruit flies and nematodes[14,2]. Recent studies have observed that a biosecure and safe way to suppress the *Aedes* mosquito species can be achieved by combining *Wolbachia* as Incompatible Insect Technique (IIT) and SIT[29]. Many pilot SIT experiments against various insect pests and mosquito species are underway, either with or without IIT[20]. Moreover, newer technologies and continuous research could enhance the cost-effectiveness of SIT application in the field.

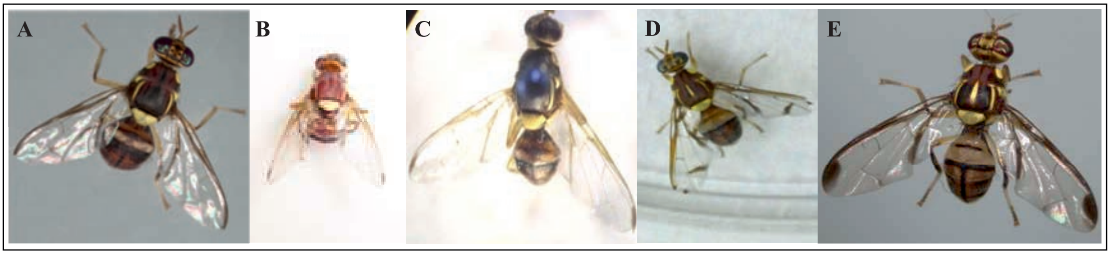

Figure 3. Economically important fruit fly species. A) The Oriental fruit fly (*B. dorsalis*), B) Peach fruit fly (*B. zonata*), C) Guava fruit fly (*B. correcta*), D) Melon fly (*Z. cucurbitae*) and E) *Z. tau*.

1.3 Improvement of sterile insect technique:

Effective and improved mass rearing procedures are the main prerequisite for consistent supply of sterile insects in large quantities. Thus, understanding the biology of target pest, developing cost effective mass rearing protocols, effective sterilisation methods and releasing techniques, improvements through modern techniques and symbionts could strengthen the successful implementation of SIT in the field. The male annihilation technique (MAT) is an environmentally benign pest control strategy. It has been observed that efficacy of SIT is greatly affected by attraction of sterile males to pheromone traps or MAT devices[5]. Thus, exploring the potential of combined/simultaneous application of SIT-MAT could improve overflooding ratios of sterile to wild male and also enhance the efficacy and cost-effectiveness of SIT.

The bacterial endosymbionts have a close interaction with insects and are known to establish various types of symbiotic associations ranging from parasitism to mutualism[7]. The bacterial communities have been profiled through classical and molecular approaches in variety of insects including fruit fly species[12,24] and their benefits have been documented (Table 2). For example, involvement of fruit fly's bacterial communities in metabolic pathways, enhances insecticide resistance, probiotics application to improve the sexual performance either at emergence or post irradiation, improvement in mass rearing, flight ability and longevity have been assessed[24,3]. Moreover, use of gut bacteria in eco-friendly control strategies for management of fruit fly species is being explored[13,3].

The accelerating development of modern biotechnology has significantly contributed in understanding the genetic information of organism. Genetic pest control strategies encompass gene editing, gene-modifications and gene-drive technology could synergistically improve the functioning of SIT. Genetic strategies can be utilised to introduce sterility-inducing genes into pest populations, which could enhance the effectiveness of SIT. CRISPR (clustered regularly interspaced short palindromic repeats) has been used to find suitable gene targets of the diamondback moth through the use of "gene drive" processes, it is possible to spread genetic constructions throughout a population that despite the construct's fitness cost provide greater-than-Mendelian inheritance[1].

Table 2. Bacterial endosymbionts associated with tephritid fruit fly species/other insects and their effects on insect biology or use in management (Source: Engel and Moran, 2013; Noman et al., 2020; Augstinos et al., 2021).

Insects	Bacteria	Effects/functions
Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann))	*Enterobacter agglomerans, Klebsiella pneumoniae, Citrobacter freundii, K. oxytoca, Pectobacterium cypripedi, Pantoea* sp.	Male-mating competitiveness, latency, longevity, receptivity, sexual calling, pupal weight, flight ability and adult size
Oriental fruit fly (*B. dorsalis* (Hendel))	*K. oxytoca, Citrobacter* sp., *Providencia, Proteus* sp., *B. cereus, R. terrigena, K. pneumoniae*	Fitness of males, improved adult emergence, male ratio, attractancy, enhances insecticide resistance; host ovaries and oviposition behaviour
Olive fruit fly (*B. oleae* ((Rossi))	*Pseudomonas putida*	Fecundity
Queensland fruit fly (*B. tryoni* ((Froggatt))	*Asaia* sp., *Enterobacter* sp., *Lactobacillus* sp.,	Shortened immature development duration
Peach fruit fly (*B. zonata* (Saunders))	*E. cloacae, K. pneumoniae*	Attractancy
Melon fly (*Z. cucurbitae* (Coquillett))	*K. oxytoca, P. agglomerans*	Attractancy
Apple maggot (*Rhagoletis pomonella* (Walsh))	*E. agglomerans*	Detoxification of plant phlorizin
Blueberry maggot (*R. mendax* (Curran))	*P. agglomerans*	Attractancy
Fruit fly (*D. melanogaster*)	*Lactobacillus* sp., *Acetobacter pomorum*	Prime immune system, affects metabolism and mating
Honey (*Apis* sp) Bumble bees (*Bombus* sp.)	*Snodgrassella alvi, Gilliamella apicola, Lactobacillus* sp.	Digestion and protection against parasites
Grasshopper (*Schistocerca gregaria* (Forskal))	*Enterococcus cloaca, Klebsiella* sp.	Help in aggregation pheromone production
Gypsy moth caterpillar (*Lymantria dispar* (Linnaeus))	*P. putida, E. faecalis, P. agglomerans*	Altering midgut permeability thereby increasing susceptibility to toxins
Reed beetle (*Macroplea* sp.)	*Macropleicola* sp.	Production of cocoon material

In order to enhance the effectiveness of current pest control methods or to develop new SIT based control systems, the above strategies can be used in conjunction with modern biotechnology tools like transcription activator-like effector nucleases (TALEN) and RNA interference (RNAi)[9]. Moreover, application of basic and molecular methods (chromosome morphologies and structural rearrangements, allozymes, mitochondrial DNA (mtDNA), microsatellites and single nucleotide polymorphisms) certainly help to plan in advance and evaluate the feasibility of SIT. Use of classical genetics and molecular approaches help in development of genetic sexing strains (GSS) in recent years which are more appropriate for SIT programme[8]. GSSs facilitate to mass produce target insects in large numbers and help in releasing of only sterile males which significantly contributes in reducing the cost of mass rearing. In recent years, the developed GSSs for certain fruit fly species (Tephritidae) and mosquitoes are being employed in SIT programme[8]. Additionally, allied technologies like Geographic Information Systems (GIS), Global Positioning System (GPS), remote sensing (RS) and spatial modelling offers a way to collect, integrate and analyze the spatial data. The above technologies are being used currently in AW-IPM programmes including SIT component[6].

1.4 Conclusions and future prospects:

The primary goal of AW-IPM is to manage the insect pest populations in the ecosystem through preventive measures. In the mid 20th century, use of ionizing radiation for insect pest control opened new branch of insect study. Continuous release of sterile insects in the field after exposure to ionizing radiation resulting in suppression or eradication of target pest. SIT is a birth control method and species specific that is becoming more popular for employment against certain key insect pest and vectors management. The efficiency of SIT in terms of genetic sexing, strain tagging, disease refractoriness and molecular sterilization will be improved by modern biotechnology and paratransgenesis. Insects are associated with diverse endosymbionts like gut bacteria and *Wolbachia* have potential to incorporate in SIT. BARC has developed SIT technologies for the management of red palm weevil, potato tuber moth and fruit flies. Further, the feasibility of SIT for red palm weevil and potato tuber moth has also been demonstrated under field conditions. Field demonstration of SIT for fruit flies is in progress. The introduction of AW-IPM programmes along with other control techniques may aid in the suppression/eradication of the insect pest population.

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