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Microbiome based insect pest management

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Abstract
Insects host diverse microbiomes comprising bacteria, fungi, viruses, and other microorganisms-that play vital roles in nutrition, immunity, development, and adaptation. Conventional pesticides, while effective, often promote resistance, harm non-target species, and cause environmental damage. In contrast, microbiome-based strategies present precise and eco-friendly alternatives by manipulating microbial partners to suppress pests or enhance beneficial insect traits. Advances in metagenomics and synthetic biology enable the identification and exploitation of insect-associated microbes with biocontrol potential. For instance, Wolbachia can be engineered or introduced to alter host reproduction and reduce vector populations such as mosquitoes. High-throughput sequencing further uncovers novel candidate microbes whose bioactive compounds or genomes may be harnessed for pest suppression or sterility induction. Symbiosis, encompassing mutualistic, commensal, and amensal interactions, underpins these associations and is fundamental to ecosystem functioning. Collectively, these approaches represent a paradigm shift toward sustainable pest management rooted in the hidden potential of insect–microbe interactions.

Keywords: Insect microbiome, symbiosis, endosymbionts, Wolbachia, metagenomics, synthetic biology, biocontrol, sustainable pest management

Introduction

Insects harbour complex microbiomes composed of bacteria, fungi, viruses, and other microorganisms that are integral to their nutrition, immune function, development, and resilience to environmental challenges. While conventional chemical pesticides have long been used to manage pest populations, their broad-spectrum activity often leads to issues such as resistance evolution, harm to non-target organisms, and environmental contamination (Rupawate *et al*. 2023) [54]. By contrast, microbiome-based tactics offer a precise, eco-friendly alternative: manipulating the microbial partners of pest species to disrupt their survival or reproduction, or bolstering beneficial insect traits to support ecosystem health.

Capitalizing on these insights, researchers are employing metagenomic surveys and synthetic biology tools to pinpoint and harness insect-associated microbes with biocontrol potential. Endosymbionts like Wolbachia, for example, can be introduced or engineered to skew host reproduction and curb vector populations such as mosquitoes. Meanwhile, advances in high-throughput sequencing reveal new candidate organisms whose bioactive molecules can be isolated or whose genomes can be edited to enhance pest-suppression efficacy or induce sterility. Together, these strategies mark a paradigm shift toward targeted, sustainable pest management grounded in the hidden power of the insect microbiome (Arora *et al*. 2018 [2]; Qadri *et al*. 2020) [50].

Symbiosis

Symbiosis is pivotal for the maintenance of the structure and functioning of ecosystems. There are different types of symbiosis such as mutualistic, commensal, and amensal symbiotic interactions, and they all are of paramount importance in all the types of ecosystems and play an essential role in organization and performance of communities. Microbial endosymbionts are microbes that are generally localized in specialized cells called mycetocytes (Costa *et al.* 1992 [16]; Szklarzewicz *et al.* 2017) [60], which often group together in a mycetome. Symbionts are usually present in the gonads of hosts and while they are also reported in the haemolymph, malpighian tubules, salivary glands, fat bodies, ovarian cells, gut and even the nervous system of their hosts (Provorov *et al*. 2018). These symbiotic bacteria are transmitted to the offspring intercellularly by the means of transovarial transmission and also extracellularly by capsule formation, jelly formation, coprophagy etc. Furthermore, insect symbionts are classified into 2 types based on the functions they perform *viz.*, obligatory symbionts and facultative symbionts. The first type is important for the insect to survive as they provide missing dietary nutrients for its survival whereas the latter ones are known to play various roles which range from protecting the insect hosts from natural enemies to the manipulation of the reproduction, male killing *etc*.

Inset endosymbionts aggravate the pestiferous activity of insects by downplaying the plant defensive responses, abiotic stress management, maintaining the insulin pathway, protecting insect host from predators, parasitoids and pathogens besides the degradation of insecticides (Montillor *et al.* 2002; Tsuchida *et al.* 2010 [62]; Soko *et al.* 2017 [58]; Handique *et al.* 2017) [30]. With this being said, it is of colossal importance to devise managerial strategies to disrupt the endosymbionts, but practically we cannot kill or suppress the symbionts at field levels but instead, symbiont mediated RNAi, whereas insect borne diseases can be controlled by using the classical technique called paratransgenesis (Durvasula *et al.* 1997; Arora *et al.* 2008 [2]; Dyson *et al.* 2022) [23].

Categories of heritable symbionts

Heritable symbionts are classified into Obligately symbiotic and Facultatively symbiotic organisms, obligately symbiotic so far as is known, they lack replicative phase or dormant phase outside hosts, but they vary as to whether they side hosts. But they vary to whether they are obligate form the host perspective, that is whether they are required for successful host development and reproduction.

These symbionts, also called primary symbionts, which are restricted to a special organ called bacteriome, which consists of distinctive host cells called bacteriocytes. Examples include *Buchnera aphidicola* in aphids, can be thought of as domesticated by hosts.

In contrast to obligate, bacteriome associated symbionts, another type is facultative symbionts, which are erratically distributed and are not required for host reproduction (Haynes *et al*. 2002 [31]; Moran *et al*. 2006) [45] Facultative symbionts resemble invasive pathogens in that they may invade various cell types, including reproductive organs, and may reside extracellularly in the body cavity (haemolymph) (Dobson *et al.* 1999 [20]) In many cases, facultative symbionts experimentally introduced to previously uninfected hosts establish stable, maternally inherited infection (Chen *et al*, 2000) [13], indicating that the persistence of the symbiosis is largely achieved through symbiont capabilities rather than host adaptations for maintaining symbiosis. In insects with bacteriomes, facultative symbionts may invade bacteriocytes where they coreside with, or even exclude, obligate symbionts (Buchner 1965 [11]). Besides retaining mechanisms for invading new hosts, entering cells, and countering host immune responses, successful facultative symbionts also must affect host phenotypes to enhance the spread and persistence of infected host lines. The nature of these effects is the basis for dividing facultative symbionts into two non-exclusive categories

Facultative mutualists confer fitness benefits upon hosts, allowing their carriers to live longer and reproduce more, thereby increasing frequencies of infected hosts. These benefits include protection against natural enemies, heat, or other mortality factors. Bacteria possess a myriad of metabolic and biosynthetic capabilities lacking in insects (and animals generally), so a wide variety of benefits to hosts are possible.

The final category, reproductive manipulators are parasites that spread by increasing host reproduction through daughters at the expense of reproduction through sons. Their strategies, which reflect the fact that heritable symbionts are usually transmitted maternally, have been reviewed extensively. One of the most common is reproductive incompatibility between infected and uninfected strains, in which infected males sterilize uninfected females, thereby increasing population frequency of infected matrilines. Other modes of reproductive manipulation are son killing (which potentially increases investment in daughters), feminization of genetic males, and parthenogenesis. The best-studied reproductive manipulator is*Wolbachia pipientis*, which is widely distributed in arthropods and some other invertebrates and which shows all of these phenotypes (Stouthamer, 1999) [59]. Reproductive manipulation has evolved repeatedly in phylogenetically diverse insect heritable symbionts, including *Cardinium hertigii* and other Bacteroidetes (Perlman, 2008) [49], *Arsenophonus nasoniae* (Enterobacteriaceae), *Spiroplasma*, and *Rickettsia* species (Perlman, 2006) [48].

Transmission of mycetocyte symbionts from parent insect to offspring

The mycetocyte symbionts are known to transmit directly from one insect generation to the next through the female. There are no known cases of insects that acquire mycetocyte symbionts from the environment or from insects other than their parents and the sole claim of paternal inheritance {In bostrychid beetles (Mansour, 1934) [40] has been discounted (Buchner, I 965) [11]. Direct evidence for exclusive maternal inheritance has been obtained in the cockroach Blattella germanica and the weevil *Sitophilus oryzae* by crossing individuals freed of their symbionts with untreated insects. In both species, the offspring of untreated females and symbiont-free males had a normal complement of symbionts but all offspring of females that lacked symbionts were symbiont-free.

The symbionts may be transmitted by the following routes

- The egg shell is smeared externally with symbionts, which are ingested by the offspring as they hatch. This mode of transmission has been reported in anobiid, cerambycid, cleonine curculionid and chrysomelid beetles and some lygaeid Heteroptera (Buchner, 1965) [11].
- The symbionts are transferred from the mycetocytes to the ovary and are incorporated into the oocytes (transovarial transmission). Most mycetocyte symbionts are transmitted by this route.

- In the viviparous Glossinidae and Hippoboscidae, the symbionts may be transmitted to larvae retained within the female reproductive tract from secretions of the milk gland on which the lavae feed (i.e., via the larval digestive tract) (Wigglesworth, 1929) [64].

	Types of transmission	Example
1	Coprophagy	Blattaria, Isoptera, Hemiptera, Hymenoptera
2	Egg and oviposition site inoculation:	Hemiptera, Coleoptera, Hymenoptera, Diptera
3	Jelly transmission	Hemiptera
4	Environmental determination	Orthoptera, Hemiptera, Thysanoptera
5	Social transmission (trophalaxis)	Isoptera, Hymenoptera
6	Capsule transmission	Hemiptera
7.	Through milk	Diptera(tsetse fly)
8.	Egg and oviposition site inoculation	Hemiptera, Coleoptera, Hymenoptera, Diptera

Different function played by endosymbionts in insects
Maintaining the insulin pathway

- Insulin like growth factor 1 pathway is well established as a critical regulator of growth and metabolic homeostasis across animal kingdom
- Insulin is key metabolic hormone that modulates carbohydrate and lipid metabolism in response to an organism nutritional state
- IIS pathway is belligerent important in aging of the insects, playing role in stress resistance.
- The insulin/IGF-like signaling (IIS) pathway is ubiquitous in multi-cellular animals and may have been involved in the evolution of multi-cellularity itself (Skorokhod *et al.* 1999) [57].

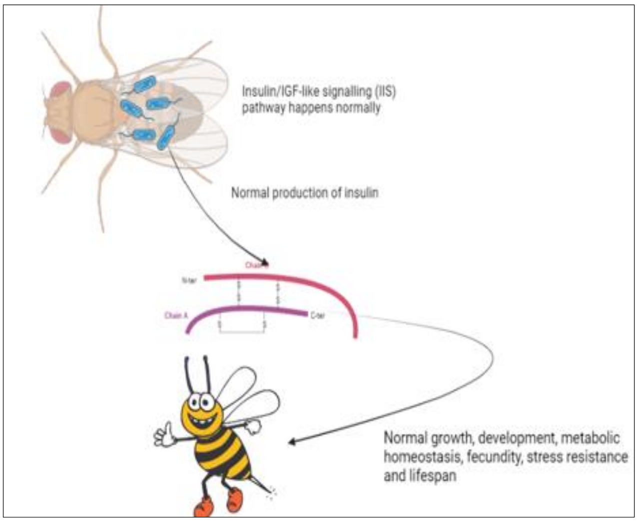
Symbiont involved in pest status of host insect

- Formation of intraspecific plant specialization by insect must have evolved through acquisition of a new food plant by a local population of the insect.
- In the case that the new food plant is an agricultural plant, the insect population will be recognized as an emergent pest.
- Traditionally, it has been believed that such ecological traits are attributed to genes encoded in the insect genomes (Feder *et al*. 1988; Hawthorne & Via 2001).
- However, recent studies have revealed that facultative bacterial symbionts may substantially affect various ecological traits of herbivorous insects.
- Recent study conducted by kikuchi *et al.* 2007(Fig: 2) showed that bacterial symbiont *Canditus* sp. Inside the insect playing vital role in pest status, and the when they transformed the symbiont from native insect i.e., *Megacopta punctatissima* to *M. cribaria* then the *M. punctatissima* became the minor pest of soyabean, furthermore, though *M. cribaria* was minor pest after getting the symbiont it started behaving as a major pest.
- The above study concluded that for insect to became an major or minor pest its decided by the type of symbiont which insect house during course of evolution.

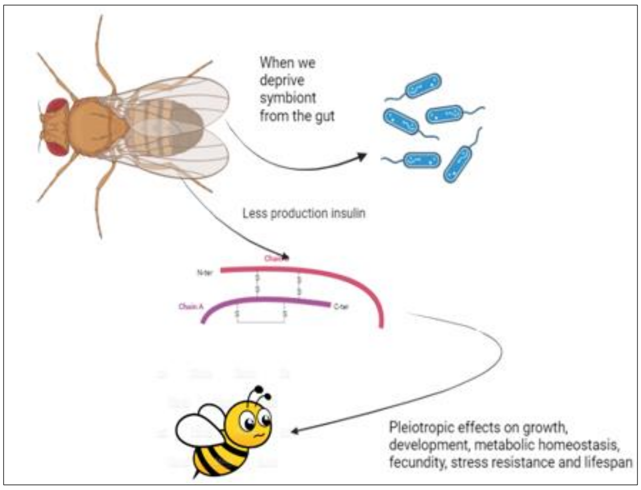
3. Role of microbial symbionts in providing missing nutrients to macro symbiont

- Several mycetocyte symbionts synthesize various nutrients required by the insect.
- In beetles and blood feeding insects symbionts known to provide B vitamins. Whereas, in cockroaches and the homopterans symbionts produce essential amino acids required for growth and development of insect, the role of symbionts in sterol nutrition of insects is uncertain.
- Symbionts help the insects to utilize nutritionally imbalanced substrates.
- Aphids cannot survive on their limited diet of plant phloem without the help of their primary symbiotic bacterium, *Buchnera*. In addition, *Buchnera* known to produce amino acid, tryptophan (rare in plant sap) to the aphid host, and also aids in the production of leucine and vitamins.
- Other sap feeding insects, such as scale insects, leaf lice, and cicadas also harbour same bacteria.
- Tsetse fly known to contain *Wiggelsworthia* as a symbiont, which synthesize vitamins that the tsetse fly does not get from the blood
- Mound-building termites and leaf-cutting ants cultivate cellulytic fungi in underground gardens, these fungi decompose the wood or leaves brought in by the termites and ants, respectively, and provide them with digestible and nutritious mycelium.
- In addition to mound, termites also harbours the bacteria in their gut which make termites digesting process easier.
- Lower termites rely on protozoa, for celluloses and hemicelluloses.
- Beetles, *Lepdiota* sp. house the flagellate protozoa, spirochaets etc. which help in degradation of cellulose. And, *Anomala* sp. house the firmicutes and proteobacteria which having cellulytic, lipholytic and nitrate reductase activity (Handique *et al.*2017) [30].

During presence of insect symbionts



During absence of insect symbionts Ikeya *et al.* 2009 [35]



Role of endosymbionts in Abiotic stress management

- Many aphids are sensitive to high temperatures. For example, some species of Sitobion fai lto reproduce at 28Cin the laboratory and pea aphids do not reproduce if subjected to a temperature of 37C for several hours as first-instar larvae.
- Rearing pea aphids for three generations at 2C also curtailed reproduction severely (Chen *et al*., 2000) [13].
- Montllor *et al.* 2002 [43] found out that pea aphids in California contain at least two facultative bacterial secondary symbionts (pea aphid secondary symbiont, PASS, or pea aphid rickettsia, PAR).
- In aphids without pea aphid secondary symbiont or pea aphid rickettsia, heat stress reduced the number of bacterio-cytes (in which the obligate primary symbiont, *Buchnera*, resides) to 7% of non-heat-stressed aphids, while aphids with only pea aphid secondary symbionts retained 70% of their bacteriocytes. Bacteriocytes in aphids with but not pea aphid secondary symbiont was reduced to 42% of controls.
- Finally, whenever there is rickettsia as secondary symbiont in aphids, they can able to manage the increasing temperature

Role of endosymbionts in production of pheromones

The congregation of locust into vast swarms can cause crop devastation of biblical proportions Guaiacol a key component of a pheromone derived from locust faecal pellets that promotes the aggregation of locusts, is produced by bacteria, *Pantoea* (Figure: 4) (Dillon *et al.* 200) [19].

Role of symbionts in protecting against pathogens.

- **Protection against nematodes:** Fruit flies, like most animals, are vulnerable to infection by a range of organisms, which, in co-infections, can interact with sometimes surprising effects. Jaenike *et al.* 2010 [36] discovered that a species of *Spiroplasma* bacterium that is sometimes found in flies, and that is transmitted from mother to offspring, protects its host from the effects of a nematode worm parasite, *Howardula aoronymphium*. The worm sterilizes the female flies and shortens their lives, but when flies were experimentally infected with *Spiroplasma*, their fertility was rescued (Figure 5).
- **Protection against fungi:** Kaltenpoth *et al.* 2005 [38] reported unique association between an new *Streptomyces* bacteria and a solitary hunting wasp, the European beewolf, beewolf known to cultivate the *Streptomyces* bacteria in the specialized antennal glands and smear them to the brood cell prior to oviposition, the bacteria taken up by the larva and occur on the walls of the cocoon. Bioassay indicate that the *Streptomycetes* protect the cocoon from fungal infection by producing the antibiotics. (Figure: 6).
- **Protection against predators:** Rove beetles used to house the symbiont, *Pseudomonas,* which produce polykide amide (pederin), this pederin makes predatory spiders to repel from the rove beetles (Piel, 2002) (Figure: 7).
- **Symbionts involved in pesticide degradation:** Chemical insecticides are used worldwide for controlling agricultural, medical, and hygienic pest insects and other organisms, which have greatly contributed to worlds agricutlture, economy, and public health. Meanwhile, indiscriminate use of insecticide leads into development of resistance in diverse pest organisms. Mechanisms underlying the insecticide resistance may involve alteration of insecticide target sites, up regulation of degrading enzymes, and enhancement of insecticide excretion, meanwhile, previous studies also showing that repeated application of insecticide cause drastic increase of pesticide degrading microbes *viz., Pseudomonas, Flavobacterium* and *Burkholderia* in agriculture field soils (Tago *et al*. 2006 [61]; Singh, 2009) [56]. These bacteria's able to hydrolyse insecticidal activity and metabolize the degradation product as a carbon source for their growth (Figure 8). Exemplified with the bean bug, *Riptorous pedestris,* known as notorious pest of leguminors crops, is associated with gut bacterial symbiont of the genus *Burkholderia* in posterior region of the midgut, in the specialized organ, these bacteria known to degrade the op compound fenitrothion into harmless compound (Figure 8).

Insecticide breakdown by diamondback moth-associated symbionts: The diamondback moth *Plutella xylostella* is a major global pest of cruciferous crops *P. xylostella* is not only able to overcome host defences, but it has even been shown to be highly resistant to a large variety of chemical insecticides and it is one of the only three insect species to have developed resistance to *Bacillus thuringiensis*-based insect control methods (Furlong *et al*., 2013) [27]. The rapid development of highly resistant phenotypes of *P. xylostella* is at least in part attributed to the insect's own physiology, and includes altered target sites for carbamates and organophosphates, metabolism of parathion via glutathione S-transferases and detoxification of pyrethroids via microsomal P-450 monooxygenases (Ramya *et al*., 2016) [51]. Isolated *Bacillus cereus* colonizing the larval gut were able to break down the insecticide indoxacarb for use in metabolism and growth (Ramya *et al*., 2015) [51]. Another insecticide, acephate, was also readily broken down by bacteria isolated from the gut of the diamondback moth.

Symbionts control: As of present players symbionts can't be killed instead we can utilize symbionts for humans own benefit by processes called paratransgenesis, symbiont mediated RNAi, etc.

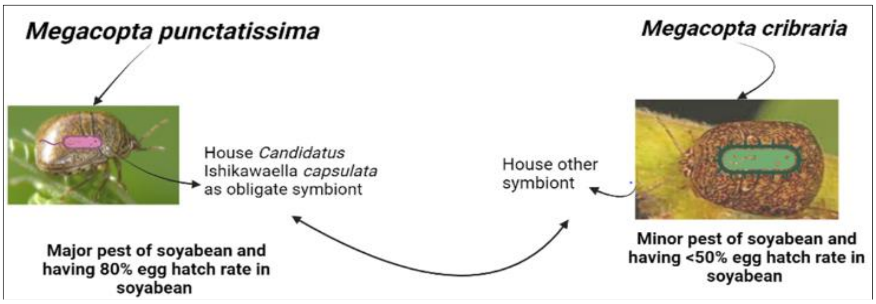


Fig 1: Relationship between soft S-metric space and contractive mappings.

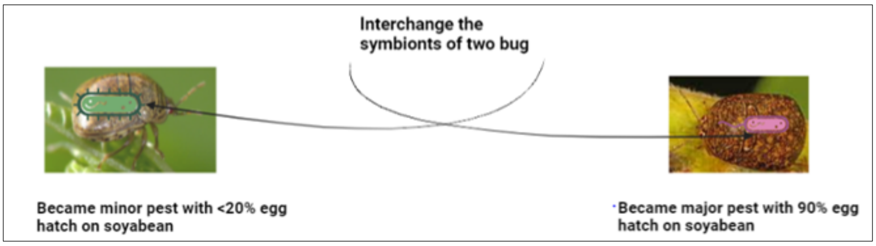


Fig 2: Stepwise process for proving a common fixed soft point.

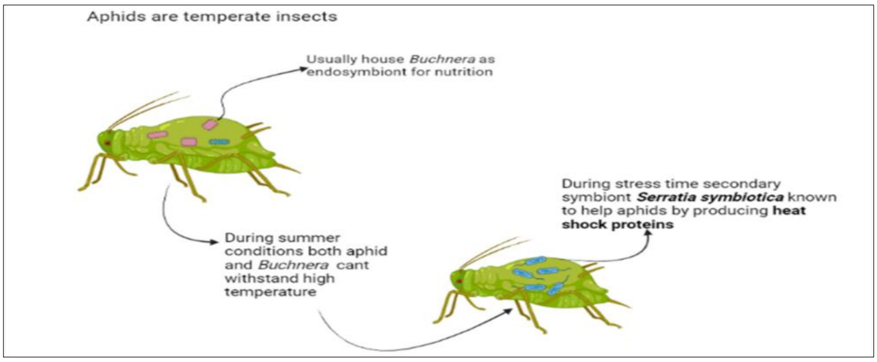


Fig 3: Example of four soft self-mappings showing compatibility.

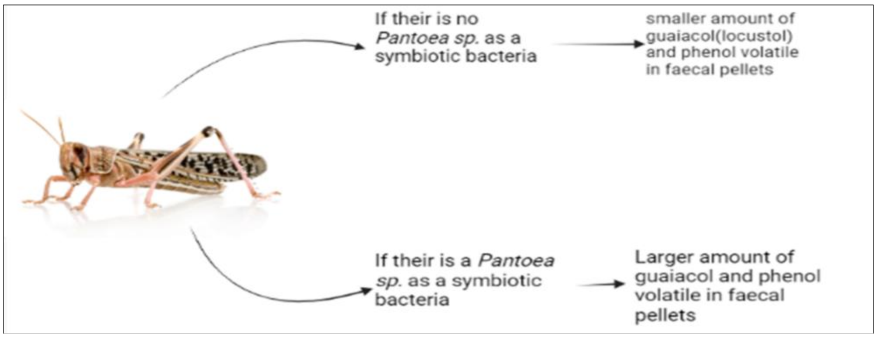


Fig 4: Corollaries illustrating special cases of the main theorem.

Role of symbionts in protecting against predators

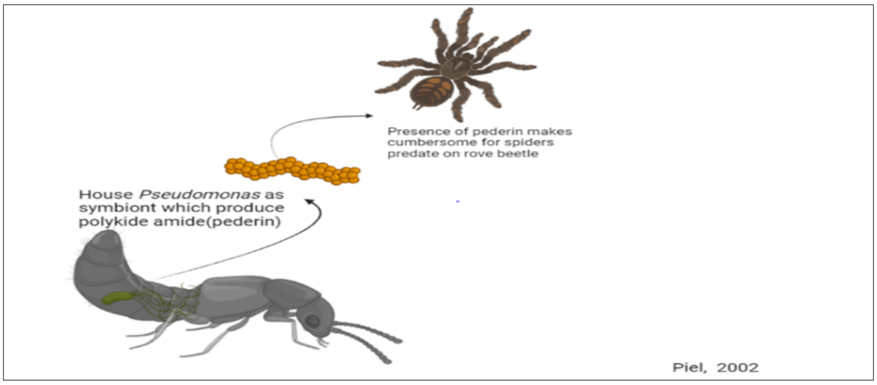


Fig 5: Flowchart of conditions leading to the existence of a unique fixed soft point.

Role of symbionts in protecting against nematodes

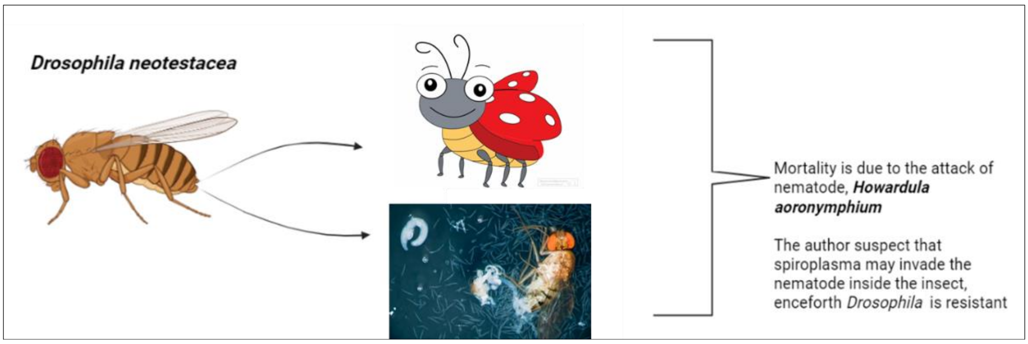


Fig 6: Convergence of soft sequences in a complete soft S-metric space.

Role of symbionts in protecting against fungi

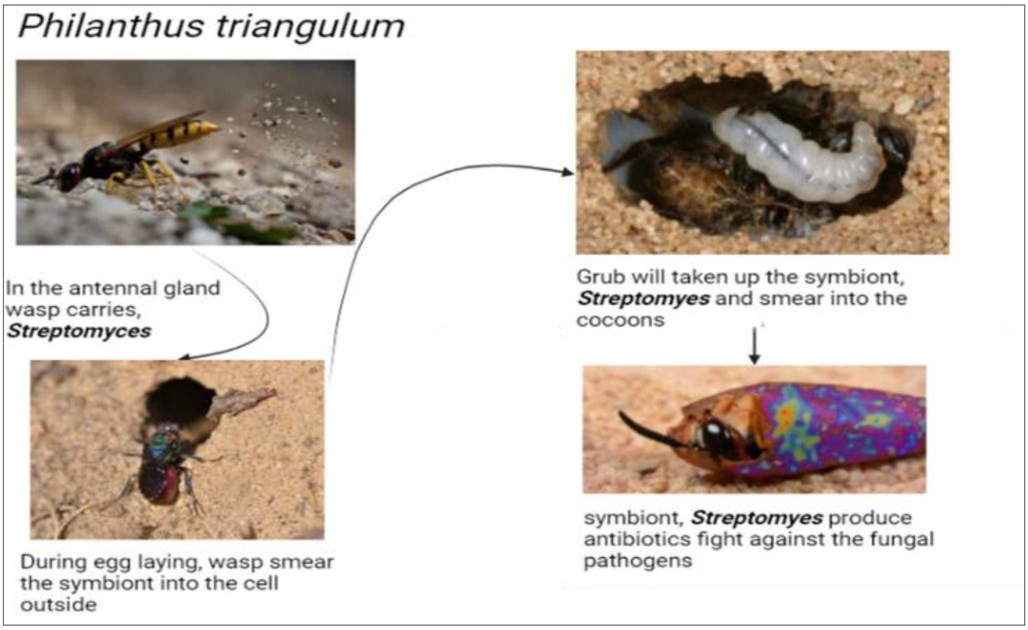


Fig 7: Graphical representation of mappings illustrating Theorem 2.3.

Role of symbionts in pesticide degradation

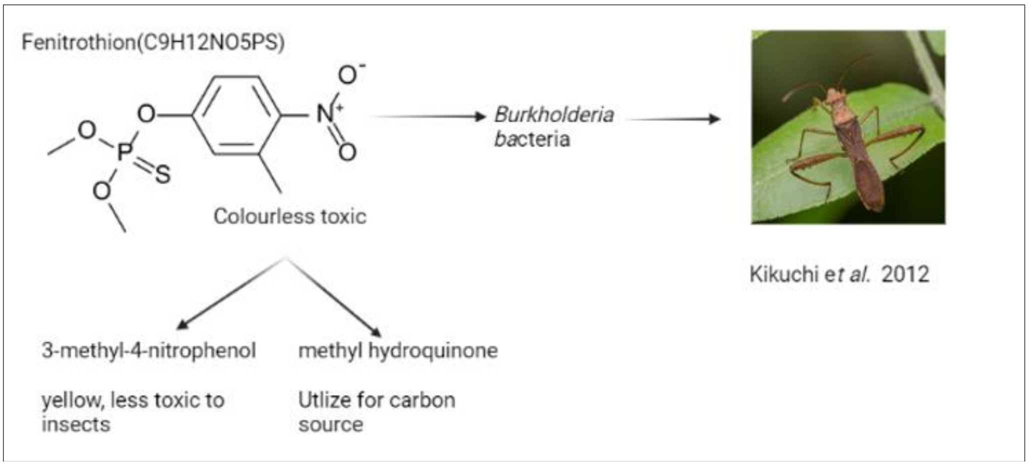


Fig 8: Demonstration of uniqueness of the common fixed soft point through contradiction.

Paratransgenesis

There was need of developing new methods to combat arthropod vector that transmit different disease in plants, humans, and animals (Ault, 1994) [3]. As concerns had raised to use insecticides for vector control and drugs for parasites control, insecticides and drugs may be used to achieve temporary reductions, but target populations often develop resistance (Georghiou and taylor, 1986 [28]. Which demands development of alternate strategies for insect vector control, one such strategy was transgenic approach where transgenic insects engineered to replace wild vector populations. However, manipulation of genome of every species of vector population to supress their capabilities to transmit pathogens is a very old scientific dream (Barik *et al.* 2020) [4]. Furthermore, a parallel approach aimed at combating vectored diseases by transformation of symbiont instead of manipulating the whole organism known as paratransgenesis. Paratransgenesis is a trojan horse approach (Beard *et al.* 2002) [5], its involved usage of recombinant symbionts to deprive the pathogens present in the vectored insects (Durvasula *et al.* 1999 [22]; Bextine *et al.* 2003 [7]; Hurwitz *et al*, 2011) [34].

Requirements for paratransgenesis

- Paratransgenesis is multidisciplinary approach it requires several requirements to be successful *viz.*,
- **Microbial ecology:** Suitable micro-organism must be identified that live in the vector in proximity to the pathogen or parasite and that can be cultured in the laboratory, the selected microbe should be associated close proximity of the targeted disease causing pathogen, this is the paramount and basic in the paratransgenesis.
- **Effector molecules:** Effector molecules can be proteins/toxins/monoclonal antibodies (Durvasula *et al.* 1997; Durvasula *et al.* 1999 [22]; Anani 2011) [1]. Effector must be inhibiting the target parasite or pathogen within the vector. Success of paratransgenesis depends on the specificity of effector molecule.
- **Effector delivery:** The effectors must be delivered from the paratransgenesis organism in efficacious concentrations and at suitable periods of time.
- **No loss of fitness cost:** Fitness cost of vectors should not harm, accordingly have to select the microbial symbiont and effector molecules.
- **Stable inheritance:** microbial symbiont carrying gene for effector molecules should be inherited stably and there shouldn't be any genetic drift.

Mechanism involved in Paratransgenesis: Paratransgenesis works on the principal of vector competence (Beard *et al.* 2002) [5], symbionts present in the insect vectors are recombined to produce effector molecules that will deprive the pathogen, further we can able to control the disease, its involved in the extraction of symbionts followed by metagenomic study will yield us the best symbiont that can be amenable for transformation process, then symbiont will be modified to express effector molecule that can eliminate only the disease causing pathogen (Figure 1), (Bextine *et al.* 2003 [7]; Arora *et al.*2018) [2].

Disease	Insect species	Pathogen targeted	Symbiont modified	Effector molecule	Reference
Chagas disease	*Rhodnius prolixus*	*Trypanosoma cruzi*	*Rhodococcus rhodni*	Cercopin-A, V_HK antibody fragment (rDB3), *Arthrobacter* β-1, 3-glucanase	Duravasula *et al.* 1997; Duravasula *et al.* 1999; Jose *et al.* 2013 [37]
Pierce diseases of grapes	*Homalodisca vitripennis*	*Xylella fastidiosa* subsp. *fastidiosa*	*Alcaligenes xylosoxidans denitrificans*	single-chain antibodies (scFv)	Bextine *et al.* 2003 [7]
Pierce diseases of grapes	*Homalodisca vitripennis*	*Xylella fastidiosa* subsp. *fastidiosa*	*Pantoeaj agglomerans*	melittin and scorpine-like molecule (SLM)	Arora *et al.* 2018 [2]
African trypanosomiasis/ sleeping sickness	Tseste fly	*Trypanosoma brucei*	*Sodalis*	Trypanocide	Medlock *et al.* 2013 [42]
Malaria	*Anopheles* Mosquito	*Plasmodium falciparum*	*Pantoea agglomerans*	SM1, anti-Pbs21, and PLA2	Bisi *et al*, 2013 [8]
Leishmaniasis	*Phlebotomus argentipes*	*Leishmania donovani*	*Bacillus subtilis*	GFP	Hurwitz *et al*, 2011 [34]

Mechanism of Paratransgenesis

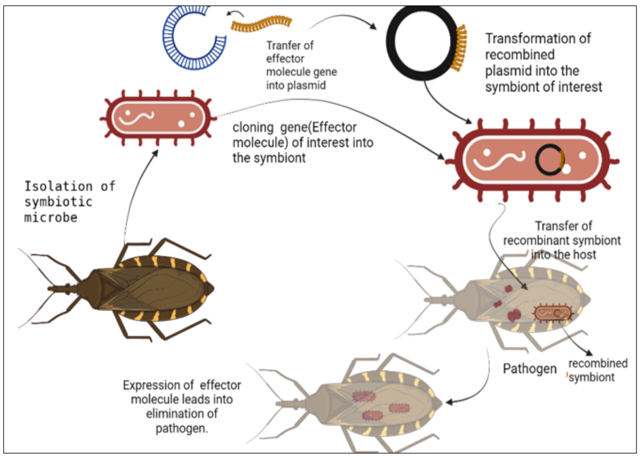


Fig 9: xxxxxxxxx

Future scope of paratransgenesis

Paratransgenesis; an insect pest control tool

Ecological important traits of insect are provided by endosymbionts, these symbionts may be obligatory or facultative, obligatory symbionts are essential for their hosts' survival and reproduction, they tend to provide missing dietary nutrients to the insects, in addition, these symbionts also affect their hosts interaction with natural enemies as well as pesticide degradation. Paratransgenesis has future scope in controlling the insect pest by inhibiting the obligatory symbionts, this can be possible by genetic modifying of facultative symbionts to express effector molecules that will deprive the obligatory symbionts in the insect pests.

Paratransgenesis; solution for insect vectored plant diseases.

Insect vector-derived plant diseases, particularly those with viral, or phloem- and xylem-limited bacterial causal agents, have great role in crop losses, as plants are immobile the epidemiology of these diseases mainly depends on their vectors capable of acquiring, persisting and transmitting of the disease causing agents (Cook *et al.* 2008) [15], as paratransgenesis strategy works on the principle of vector competence, moreover, Bextine *et al.* 2003 [7]; Arora AK, 2015 had successfully inhibited the *Xylella fastidiosa* causing the grapevine pierce disease, this provides an array of scope and confidence for utilizing the paratransgenesis platform for the control of plant disease vectors.

Conclusion

Paratransgenesis is a Trojan horse concept, had started with inhibiting the vector borne human disease, and in a half decade it had spread into the vector borne plant disease, although paratransgenesis on plant disease got succeed in inhibiting *Xylella fastidiosa* causing grapevine pierce disease, but still paratransgenesis on other vector borne plant disease have not been tried, so in future paratransgenesis on plant diseases as well as for improving the colony health of honey bees, improving the commercial traits of silkworm and in insect pest management paratransgenesis can be seen.

Symbiont mediated RNAi

RNAi: The main antiviral immune system of insects is the post transcriptional gene silencing mechanism known as RNAi. This, post transcriptional mechanism of silencing gene function by inserting short homologous sequence of messenger RNA (mRNA) to prevent translation of proteins.

Mechanism of RNAi

The RNA precursor molecules from the RNAi pathways in insects are identified as small RNAs. These are of three types *viz.* small interfering RNAs (siRNAs: 20-25 nucleotides) microRNAs (miRNAs: 21-24nucleotides) and the PIWI-interacting RNAs (piRNAs;24-30 nucleotides). Both miRNAs and siRNAs share a common RNAse-111 processing enzyme, Dicer. While piRNAs are independent of Dicer activity. In the siRNA pathway, the dsRNA is processed by Dicer into siRNA duplexes. But the miRNAs are generated from endogenous transcripts in nucleus as pre miRNAs. It is then processed by Enzyme Drosha and finally transported to cytoplasm. Both these RNAs contain ribonucleoprotein particles (RNPs). The siRNAs contain RISC "RNA induced silencing complex" and a miRNA contain miRNPs. Every RISC or miRNPs contains a member of the Argonaute (AGO) protein family. The AGO protein uses the guide RNA to associate with target RNAs and then silencing of the target mRNA occurs. In most cases a single Dicer is responsible for both the siRNA and miRNA pathways. While in *Drosophila melanogaster* has two prologues, one is Dicer 1 which process miRNAs and other Dicer2 which process siRNAs. In drosophila, AGO-1 is involved in the miRNA pathway and AGO-2 in the siRNA pathway. Although in *Tribolium cataneum* only one type of AGO protein has been identified in miRNA (i.e., Tc Ago1) and two classes of AGO protein pathway in siRNA. Whereas in *Bombyx mori* three RNAi pathways has been identified (Kolliopoulou *et al.*, 2014). As such RNAi has become the most widely used reverse genetics research tool in insect and have great potential to contribute to novel strategies for species specific control of insect pests and to overcome viral infections in disease vectoring and beneficial insects.

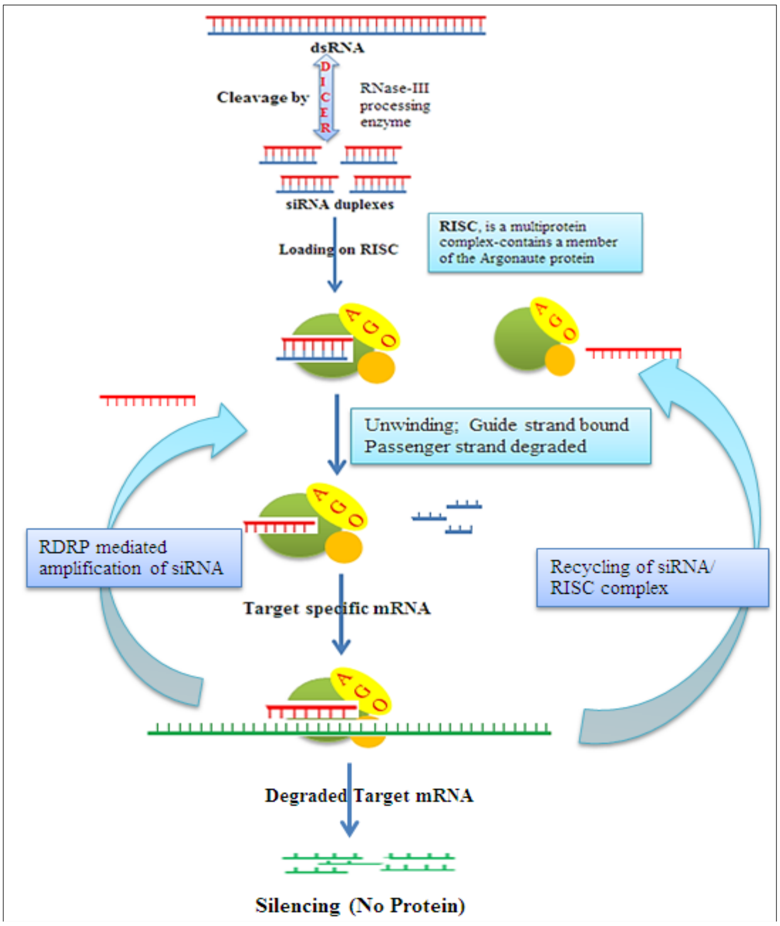


Fig 10: xxxxxxxxx

Symbiont mediated RNAi: Symbiont mediated RNAi is an novel way to exploit the association between culturable symbiotic gut bacteria and their hosts: to constitutively deliver dsRNA to evoke RNAi in the host. The technology provides a feasible means both to investigate gene function and as a biocide to control insect population size. The cumbersomeness of using RNAi in insects are *viz*., insect lack the RNA-dependent RNA polymerase enzyme, RNAi is normally delivered by transgenic plants, meanwhile Dyson *et al*. 2022 [23] said that insect do have salivary enzymes which degrades the RNAi, so all this intricates can be resolved.

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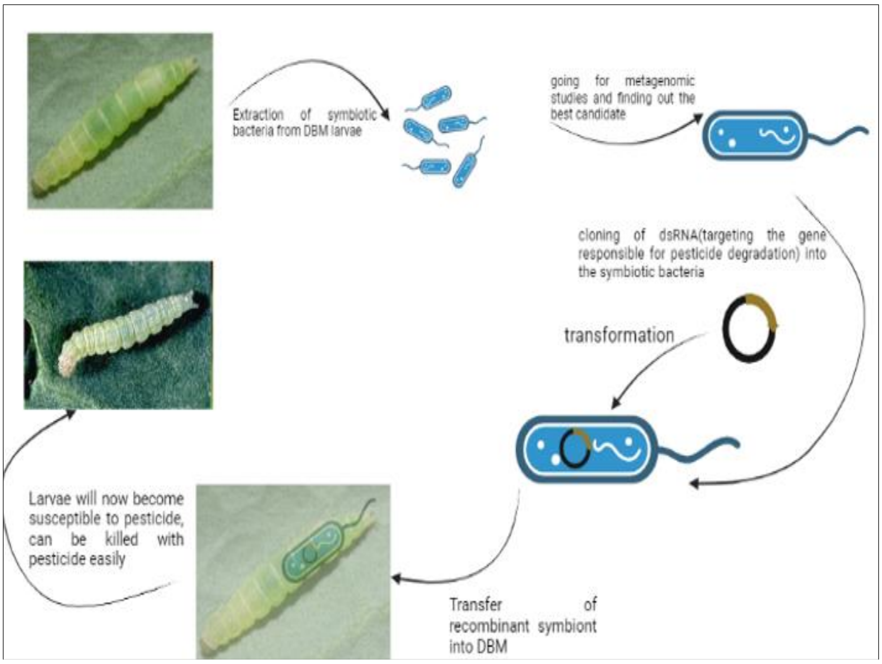

Fig 11: xxxxxxxxx

Dysbiosis in insect pest management

The growing need for sustainable pest control has spotlighted *dysbiosis* the deliberate disruption of insect-associated microbial communities as a promising alternative to chemical pesticides. Insects rely heavily on their microbiota for digestion, immunity, and detoxification; for example, *Drosophila* depends on gut bacteria to metabolize nutrients (Douglas, 2015) [21], while termites use symbiotic microbes to break down cellulose (Engel & Moran, 2013) [24]. By destabilizing these partnerships, researchers can impair pest survival: antibiotics reduce microbial diversity in pests like the Colorado potato beetle, weakening their defenses (Cheng *et al*., 2017) [14], while introducing harmful bacteria (e.g., *Serratia marcescens* in fruit flies) disrupts beneficial symbionts (Ratzka *et al*., 2013) [52].

One of the most promising tools for inducing dysbiosis is the use of antibiotics. When administered to pests like the Colorado potato beetle (*Leptinotarsa decemlineata*), antibiotics reduce microbial diversity, stripping the insect of its ability to detoxify plant defenses or synthetic pesticides (Cheng *et al*., 2017) [14]. Similarly, dietary interventions such as altering nitrogen availability can starve out essential symbionts in aphids (*Acyrthosiphon pisum*), causing stunted growth and population collapse (Wilkinson *et al*., 2007) [65]. Modern technologies like RNA interference (RNAi) add precision to this approach. In diamondback moths (*Plutella xylostella*), suppressing genes critical for microbial symbiosis rendered the pests vulnerable to *Bacillus thuringiensis* (Bt), a naturally occurring biopesticide (Xia *et al*., 2021) [66]. Even sterile insect techniques, used to control invasive fruit flies (*Ceratitis capitata*), have been enhanced by manipulating gut bacteria to reduce mating competitiveness. These examples underscore dysbiosis's versatility, offering tailored solutions for diverse pest species.

Case studies highlight successes: aphids deprived of *Buchnera* bacteria fail to synthesize essential nutrients (Wilkinson *et al*., 2007) [65], and mosquitoes with depleted microbiota become easier targets for malaria control. However, challenges like off-target effects on non-pest species (Mason *et al*., 2021) [41] and ecological ripple effects demand caution. As microbiome research advances, combining dysbiosis with integrated pest management could offer a safer, greener future for agriculture if deployed thoughtfully.

However, the approach is not without challenges. Specificity remains a major hurdle many interventions risk harming beneficial insects or soil microbes. For example, broad-spectrum antibiotics might inadvertently decimate pollinators like bees or disrupt nutrient-cycling bacteria in ecosystems (Mason *et al*., 2021) [41]. Additionally, pests could evolve resistance by recruiting alternative microbial partners or evolving compensatory traits. Ecological ripple effects are another concern, removing a pest species might destabilize food webs or inadvertently favour secondary pests. Ethically, large scale manipulation of insect microbiomes raises questions about unintended consequences, akin to debates over genetically modified crops.

Despite these challenges, the future of dysbiosis in pest management is bright. Advances in microbiome sequencing allow scientists to identify keystone microbial species that are critical for pest survival, enabling highly targeted interventions. Engineered probiotics, designed to outcompete beneficial microbes or deliver toxins, could offer species-specific control. Phage therapy, which uses viruses to attack pest-associated bacteria, is another precision tool under exploration. Integrating dysbiosis with existing strategies like crop rotation, biological control agents, or pheromone traps could create synergistic effects, reducing reliance on any single method. For instance, combining dysbiosis-inducing RNAi sprays with Bt crops might delay resistance evolution while minimizing chemical use. While challenges like off-target effects and resistance loom, the fusion of cutting-edge science and ecological wisdom offers a path forward. As we refine these tools, dysbiosis could help cultivate a future where farms thrive, ecosystems rebound, and chemical pesticides fade into obsolescence a vision as hopeful as it is urgent.

Cytoplasmic incompatibility (CI) and Incompatible Insect Technique (IIT)

Cytoplasmic incompatibility (CI), a reproductive phenomenon mediated by the intracellular bacterium *Wolbachia*, has emerged as a groundbreaking tool in sustainable insect pest management. CI occurs when *Wolbachia* infected males mate with uninfected females or females harbouring incompatible *Wolbachia* strains, leading to embryonic lethality due to chromosomal segregation defects. This mechanism has been harnessed through the Incompatible Insect Technique (IIT), a species-specific approach where mass reared, incompatible males are released to suppress wild pest populations by inducing sterility in wild females. Unlike traditional methods like chemical pesticides, IIT minimizes environmental harm and resistance risks while preserving non-target organisms (Werren *et al*. 2008 [63]; O'Neill, 2018) [46]. For instance, IIT has shown remarkable success against *Aedes aegypti* mosquitoes, vectors of dengue and Zika viruses, by reducing population densities in field trials across regions like Australia and Southeast Asia (Hoffmann *et al*., 2014 [32]; Zheng *et al*., 2019) [69]. Similarly, applications in fruit flies (*Ceratitis capitata*) and disease-transmitting tsetse flies (*Glossina spp.*) highlight its versatility (Zabalou *et al*., 2004 [67]; Bouyer *et al*., 2020) [10]. Challenges remain, such as ensuring the exclusive release of non-transmitting males to prevent accidental establishment of incompatible *Wolbachia* strains which could undermine efficacy. Advances in sex-sorting technologies and combining IIT with radiation-induced sterility (e.g., "combined SIT/IIT") have mitigated this risk (Zhang *et al*., 2016) [68]. Moreover, IIT's integration with other biocontrol strategies, such as gene drives or pathogen-blocking *Wolbachia* strains, offers synergistic potential for long-term pest suppression and disease control (Carvalho *et al*., 2015) [12]. Despite logistical hurdles like cost and scalability, IIT represents a paradigm shift toward ecologically responsible pest management. Its success hinges on interdisciplinary collaboration, community engagement, and adaptive field testing to refine protocols across diverse agroecological contexts. As resistance to conventional pesticides escalates, IIT stands out as a beacon of innovation in balancing agricultural productivity and environmental stewardship.

Microbial semiochemicals in insect pest management

Microbial semiochemicals (chemical signals produced by microorganisms) are emerging as powerful tools in sustainable insect management, offering eco-friendly alternatives to conventional pesticides. These compounds, integral to insect communication, influence behaviors such as mating, foraging, and predator avoidance (Dicke and Sabelis, 1988) [18]. Microbes like bacteria, fungi, and yeasts emit volatile organic compounds (VOCs) that can attract or repel pests, enabling targeted control strategies. For instance, Davis *et al*. (2013) [17] identified bacterial volatiles that lure fruit flies (*Drosophila melanogaster*), suggesting their use in bait traps. Conversely, yeast-associated compounds disrupt mosquito host-seeking behavior, potentially reducing disease transmission (Rering *et al*., 2018) [53]. Microbial symbioses further highlight their utility: bark beetles rely on symbiotic fungi to produce aggregation pheromones, a vulnerability exploited to disrupt infestations (Boone *et al*., 2008) [9]. Similarly, fungal metabolites interfere with the mating behaviors of pests like the coffee berry borer, *Hypothenemus hampei*, by masking pheromone signals (Hulcr *et al*., 2020) [33]. Goelen *et al*. (2020) [29] demonstrated that bacterial volatiles, particularly a synthetic blend of styrene (1 µg) and benzaldehyde (10 ng), effectively attract the aphid parasitoid *Aphidius colemani* in laboratory and greenhouse experiments. The blend outperformed individual compounds and bacterial culture media, achieving a 75 per cent parasitoid preference in lab assays and significant attraction in greenhouse trials, suggesting its potential as a targeted biocontrol tool. This study highlighted microbial semiochemicals as a sustainable strategy to enhance natural enemy retention in crops, advancing eco-friendly pest management.

The advantages of microbial semiochemicals lie in their specificity and minimal environmental impact, avoiding harm to non-target species. However, challenges such as field stability, large-scale production, and ecological complexity hinder widespread adoption. Innovations in synthetic biology and microbial consortia engineering promise solutions, enabling tailored semiochemical production. Integrating these compounds into integrated pest management (IPM) systems could revolutionize agriculture, reducing reliance on toxic chemicals. As research advances, microbial semiochemicals may unlock precise, sustainable pest control methods, aligning agricultural practices with ecological balance.

Conclusion

In-depth studies of microbiomes across diverse agro ecosystems such as those found in insects, plants, and other natural resources are paving the way for exciting discoveries. This era holds great promise for identifying new microbes or microbiome functions that could be harnessed for controlling insect pests. Advances in state-of-the-art technologies like gene editing, microbial engineering, and nanotechnology are helping scientists improve methods for extracting bioactive compounds from microbes that can't be cultured in the lab. These breakthroughs are expected to play a crucial role in driving agricultural innovation and more effective pest management strategies.

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