



A critical review on Arthropods pests' management of major crops

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Abstract

Phytophagous arthropods are a challenging problem in agriculture, horticulture, and forestry. Various cultural practices and the use of resistant plant varieties are among the traditional pest control methods but until recently the application of synthetic acaricides and insecticides had been the main solution. Due to side effects of the anti-insectan chemicals (for instance, on insect pollinators or parasitoids) and environmental pollution, the search for safer methods of controlling pest arthropods has been underway. The biological method of insect control based on the use of their parasites, predators, and pathogens (viral, bacterial, nematode, and fungal) is becoming increasingly accepted, especially in organic farming. However, efficacy of biocontrol agents is highly depended on number abiotic and biotic factors and often insufficient when compared to chemical control. Some RNAi-based products are already available for farmers and more are expected to reach the market soon. Tailor-made dsRNA as an active ingredient for biopesticide formulations is considered a raw material that can be used for diverse purposes, from pest control and bee protection against viruses to pesticide resistance management. The RNAi mechanism works at the messenger RNA (mRNA) level, exploiting a sequence-dependent mode of action, which makes it unique in potency and selectivity compared with conventional agrochemicals. Furthermore, the use of RNAi in crop protection can be achieved by employing plant-incorporated protectants through plant transformation, but also by non-transformative strategies such as the use of formulations of sprayable RNAs as direct control agents, resistance factor repressors or developmental disruptors.

Key words: RNAi, arthropods, agriculture, crop protection

Introduction

Pest management has played a critical role in achieving the current level of the global food supply, and its importance will only increase in the future. With the world population predicted to reach 9 billion by 2050, and one in seven of the current population still without access to sufficient nutrition, growth in the food supply cannot decelerate. Greater demands for meat and dairy that accompany rising incomes, along with a potentially tremendous expansion in the use of biofuels, will put additional pressure on agricultural output. However, options for increasing output are more limited than they were in the past. The scarcity of water and land, along with climate change and large-scale biodiversity losses, makes significant expansion of farmland infeasible. Evidence is also beginning to suggest that growth in maximum potential crop yields may be slowing. Efficient pest management toward maximizing actual yields on existing farmland is therefore of critical importance (Zafar et al., 2020; Zafar et al., 2022). Crop loss assessment is a necessary prerequisite for a pest management program. By comparing the value of a reduction in crop production or quality with the cost of limiting or preventing the loss, an informed decision may be made by the grower as to when, where, how, or whether to apply control measures. For the policy maker, such information permits limited resources to be concentrated on those problems of significant economic importance. In particular, accurate data on losses to pests are essential to establish the economic injury level, the threshold of pest density or disease intensity, above which control becomes necessary to prevent economic damage, and to provide a basis for the evaluation of costs versus benefits in the application of control measures. As part of minimizing the risk of crop losses due to herbivorous arthropods (here, mainly referring to insects and mites), most of the World's commercial food production systems are subjected to several applications of pesticides before being harvested (Guedes et al., 2016). Some crops are sprayed 10-20 times, while most field crops are sprayed 1-5 times during the growing season. In the US alone, the average number of insecticide applications ranges from 1-3 for most of the major field crops, and the total annual costs of pesticide applications exceed \$1.3 billion. Crop loss assessment has three general components: detection of the harmful organism; measurement of the extent of the infestation or infection; and evaluation of the response of the crop to infection or infestation. IPM was conceptualized during the 1950s when insecticide resistance, non-target effects, and economic waste were clearly apparent. Practitioners understood the consequences for the larger environment and the longer term, but implementation of IPM emphasized short-term economic efficiency, and integration of cultural, biological, and chemical control measures. For example, by including natural enemies as biological control agents in the management of pests, IPM practitioners knew that more specific and less harmful chemicals would need to be used over the long run. Chief among pests responsible for the unacceptably high losses in crops are arthropods, and these losses have been increasing over recent decades (Razzaq et al., 2021). Insects and mites account for the majority of the damage to crops, with a much smaller proportion attributable to other groups, such as the Collembola, Symphyla, and Oniscidea. Among the mites, the worst pests are found in the orders Trombidiformes (families Tydeidae, Phytoseptidae, Ditylomiopidae, Eriophyidae, Tetranychidae, Tenuipalpidae, Tuckerellidae, and Tarsonemidae) and Sarcoptiformes (Acaridae) (Jeppson et al. 1975; Krantz and Walter 2009). The Orthoptera, Hemiptera (Heteroptera and Homoptera), Thysanoptera, Coleoptera, Lepidoptera, and Diptera are considered the most important insect

orders containing agricultural pests. Recent years have seen a growing recognition of the importance, as pests of stored grains and other food products, of the Psocoptera (book and bark lice), a group hitherto considered of no significance in that context. However, although they are the major destroyers of crops, probably fewer than 1% of all insect species may be considered pests in any way, and, of these, perhaps 3500 species require regular attention (Guedes et al., 2014). Arthropods have been consuming the tissues of living green plants since the Late Silurian Period, about 416 million years ago. In natural ecosystems, this herbivory tends to be minor, amounting to 0.5–15% of net plant (primary) production, and usually does not impair overall plant productivity. However, the conversion of natural systems to arable agriculture brings arthropod feeding into conflict with human interests, and puts crop production at risk. In their simplified structures, open mineral cycles, and high rates of biomass accumulation (high yields), agroecosystems resemble the early stages of ecological succession. That description applies to modern, industrialized agriculture, in particular, with its tendency towards single-stand cropping and extensive dependence on external inputs. Crop losses due to insect pests have been reported to be greater under modern than under traditional agricultural practices. This is thought to result from the greater spatial & temporal complexity of traditional agriculture, which fosters conditions conducive to increases in pest populations. The reduction in plant species richness brings about changes in the composition of the resident pest community, and typically results in greater crop losses from a pest complex that is less diverse, but more abundant, and dominated by specialist herbivores with a narrow host range (Zehnder et al., 2007). Also, the myriad stresses and imbalances, to which crop plants are subjected under modern production systems, are thought to lower their resistance to pests. In particular, the increased crop productivity characteristic of modern agriculture, an advance made possible by the “green revolution,” often is associated with higher vulnerability to pest attack. Arthropods damage crops in a variety of ways. They attack growing plants by: chewing leaves, buds, stems, bark, or fruit; sucking the sap from leaves, buds, stems, or fruit; boring into or tunneling through the bark, stems, or twigs, into fruit, nuts, or seeds, or between the surfaces of leaves; causing cancer-like growths on plants (galls), within which they live; attacking roots or underground stems in any of the above ways; laying eggs in plant parts; taking plant parts for the construction of nests or shelters; and transmitting plant pathogens. They destroy or depreciate the value of stored plant products by: consuming the items as food; contaminating them with their secretions, fecal material, eggs, or their own bodies; and increasing the labor and expense of sorting, packing, and preserving foods (Safdar et al., 2022). Fairly detailed figures on crop losses to insect and mite pests, in monetary terms, were compiled and published by the U.S. Department of Agriculture during the previous century, several compilations appearing in the Department’s Cooperative Economic Insect Report and the succeeding Cooperative Plant Pest Report series. In an early report, overall losses in the United States were estimated to range from 10–20%. Recent estimates of the amount of pre-harvest crop production destroyed globally each year by arthropods, incorporating the earlier-published data, similarly are on the order of 13–16%. Assuming the worst case (percentage losses at the higher end), and based on current figures for production values of the most important cereals and other crops, economic losses due to crop-destroying 208 T. W. Culliney arthropods exceed \$ 470 billion annually, undoubtedly an underestimate, given the paucity of data from the developing world.

Host–Microbe Interactions

Microbial associations with invertebrates are common, widespread and show large variation in their community diversity, fitness impacts/effects and routes of transmission. These characteristics are interlinked and fundamental to our understanding of host-microbe associations as these are the qualities that shape the ecology of microorganisms in association with their hosts. However, most of this diversity is not amenable to culture in the laboratory making the study and manipulation of these relationships difficult. The use of molecular techniques to identify non-culture viable microorganisms has allowed research to accurately classify organisms previously only described by drawings and micrographs (Butt et al., 2016). The ability to name and identify these bacteria has started to uncover a fascinating diversity of organisms that have a long and complex evolutionary history with their insect partners. An evolutionary perspective on microbial host interactions has aided our understanding of how important microorganisms are to insect biology. The ubiquitous presence of particular bacterial in association with an insect genus or group indicates that the bacteria may have coevolved with the insect ancestors for millions of years. The occurrence of very closely related bacteria in unrelated or distantly related host species may indicate that the bacterium is frequently transmitted between species and good coloniser of insects (Saldaña et al., 2017). This evolutionary perspective is very powerful, but it cannot tell us the details of the current relationship that a microbe has with its host. Pathogens (harmful or deleterious to the insect host), commensals (neutral to the host) and mutualists (beneficial to the host) all show parallel patterns of evolutionary association with their hosts so experimental work is often needed to determine the precise nature for the relationship. In practice some microbes may possess different associations depending on the physiological state of the host. Importantly, an evolutionary overview can highlight long term and persistent relationships; but it is likely that a number of microbial relationships with the host are transient and/or opportunistic. Therefore, to understand the relationship and the role of these micro-organisms in the host we must study the impact they have on the insect’s performance and fitness (Sanchez-Contreras et al., 2008).

Friendly Bacteria Many bacteria have co-evolved with insects to be exclusively mutualistic, typically these bacteria have long evolutionary histories with their host insect, e.g. *Buchnera* species in aphids and *Wigglesworthia* species in tsetse flies. In these relationships the symbiotic bacteria provides nutritional elements that are absent from the insects diet and the bacteria is essential to the fitness and reproductive success of the insect. This type of specialised mutualistic relationship is very important to insect performance, but the more common situation is likely to be that the insect’s microbiota is generally commensal showing transient positive and negative effects of the insect’s performance. One example is the mutual relationship of the desert locust with its resident gut microbiota. The beneficial effect of the microbiota is only evident either during periods of starvation or during the exhibition of certain behavioural traits by the insect host. These relationships can be very stable over evolutionary time, but can equally be short lived and ephemeral. This diversity of microbial interactions with hosts makes defining relationships between an insect and its microbiota difficult, as the outcome of the interactions may vary with circumstance and the length of their evolutionary association. However, we can define some types of symbiotic relationships quite precisely based on their evolutionary biology. Symbiont diversity can be summarised into three broad classes: (1) the Mycetome or bacteriome-associated obligate symbionts (transferred, relatively recent origin of symbiosis (~2 million years) and clusters of closely related taxa across multiple hosts. These different classes of symbiotic relationship lend themselves to different distinct and targeted applications, due to their importance to the insect biology, transmissibility and mode of interaction with the insect (Biedermann et al., 2017).

Soil Quality

Management Proponents of organic farming have long promoted the view that the likelihood of pest outbreaks is reduced with organic farming practices, including the establishment and maintenance of “healthy” soil. Within this context, organic or ecologically based pest management considers belowground and aboveground habitat management equally important. In organic farming, enhancement of soil fertility is accomplished through rotations, cover cropping, and the application of plant and animal materials. Recent studies have shown that plant resistance to insect and disease pests is linked to optimal physical, chemical, and, perhaps most importantly, biological properties of soil. Several researchers have reported lower numbers of pest insects on crops grown with organic compared with synthetic sources of fertilizer (Roy et al., 2018). In paired comparisons of soil from organic and conventional farms, experimentally partitioned fertilizer source and soil management history effects influencing the European corn borer (ECB), *Ostrinia nubilalis*, host preference for corn. They demonstrated a lower level of ECB oviposition on corn grown in organically managed soil and suggested that the observed pattern of ECB egg laying resulted from a form of biological buffering as a result of organic-soil management. Subsequent experiments supported this mineral balance hypothesis and suggested that the organic matter and microbial activity associated with organically managed soils provide a buffering capability to maintain optimal nutrient and mineral balance in plants, which in turn affects the performance of phytophagous insects (Gonçalves et al., 2020). Potato grown in manure-amended soils was an inferior host for the Colorado potato beetle compared with potato grown in synthetically fertilized soil. Organic mulches are often used in organic farming to add organic matter to soil and to increase soil-moisture-holding capacity and reduce soil temperature. Studies have shown that application of straw mulch can suppress some insect pests such as the Colorado potato beetle, probably through a combination of effects involving reduced host-finding ability and increased predation from natural enemies. Straw mulch has also been well studied in reducing aphid infestation and virus incidence in several crops. However, development of some pests such as the squash bug, *Anasa tristis*, and the American palm cixiid, *Myndus crudus*, are favored by application of organic mulch. Soil arthropod diversity, distribution and abundance are determined by several factors such as organic matter content, soil features, cover vegetation, soil disturbance (mechanical soil tillage), fire, and pollution. Moreover, the different taxa respond differently to a variety of environmental factors. Some groups are more sensitive, while others are ubiquitous and more able to react to soil degradation. In addition, different species belonging to the same taxonomic group can respond differently. Because of this complex scenario, the subject is particularly complex, as found in a wide number of papers published on this topic. As is known, Acari and Collembola are the two groups which are generally considered in soil quality evaluation approach. Other groups, such as Coleoptera, Diptera and Araneae, are often involved in studies aimed to evaluate soil quality/degradation/pollution, while other groups, typically Symphyla, Paupoda, Pseudoscorpionida and others, are generally little discussed in soil quality monitoring or discussed together with other soil arthropods taxa. Finally, the use as bioindicator in soil monitoring programs is still missing for some groups, such as Palpigrada. In our opinion, further studies are needed to develop the ecological knowledge of the more sensitive groups and understand how the whole soil arthropod community is able to react to soil perturbation and recover (Menta, & Remelli et al., 2020).

Soil Fungi

Micromycetes of the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, and others are most often isolated from soil samples. Sometimes the fungi have been isolated from died insects and can even be causal agents of their mycoses. For instance, the virulence of *A. flavus* against *G. mellonella* larvae, *F. larvarum*, *F. proliferatum* and *T. harzianum* against aphid *S. graminum*, *F. solani* and *T. harzianum* for the American cockroach *Periplaneta americana*, *F. subglutinans*—the Western flower thrips *Frankliniella occidentalis* *Aspergillus* and *Fusarium* spp.—against the silverleaf whitefly *Bremisia tabaci* was proved. Since soil fungi have long been known as producers of biologically active compounds, one can assume their toxigenic effect on arthropods. On the other hand, their insecticidal metabolites can be considered as protection factors against mycetophages, since they mainly accumulate in sclerotia (resting mycelial modifications), ascostroma, or in vegetative mycelium post its mechanical damage. Fumagillin, gliotoxin, and ergoalkaloids have been shown to play an important role in the infection of *G. mellonella* larvae with *A. fumigatus*. Further, significant differences were noted in the qualitative composition of extracts from sclerotia and mycelium of some aspergilli. From the resting structures of *Aspergillus* spp., about 100 new compounds were isolated, most of which demonstrated anti-insectan activity. A number of them had been patented as potential insecticidal molecules: aspernomine, sulpinine, secopenitrem B, aflatrem B, leporin A, cycloechinulin (Berestetskiy & Hu, 2021).

Trap cropping

In organic crop production, pest management relies primarily on habitat manipulation through farm scaping and other biological control practices. It has been observed that polycultures of crop species often lead to less damage from pests than monocultures of crops within a given area. One explanation for this was proposed by Root, that polycultures can enhance biological control by offering greater host capacity for natural enemies while simultaneously complicating the pest habitat. A habitat manipulation through trap cropping capitalizes on the strong perimeter-driven behavior in multiple cropping systems.

Trap cropping is an attractive option to reduce dependency on conventional pest management practices through insecticides. Indeed, insecticides are costly and hazardous (even the organic ones), and some insect pests have developed resistance against them. An example could be the stink bug (Hemiptera: Pentatomidae), which can further be exacerbated by their long life cycle, high capacity to disperse and polyphagous nature leading to a landscape-wide agro-ecosystem threat. Trap crops have been shown to effectively manage stink bugs (*Halyomorpha halys* Stål (Hemiptera: Pentatomidae)) in conventional and organic crop production systems. Sorghum (*Sorghum bicolor* L.) has been successfully used as trap crop in cotton fields. Similarly, black mustard reduced kernel injury by 22% in sweet corn caused by *Nezara viridula* L. (Heteroptera: Pentatomidae). An efficient trap crop system should have at least double the pest attraction capacity of the cash crop during its vulnerable stage with an easy management strategy and should cover no more than 2%–10% of the total crop area.

Phytopathogens

There are few reports of anti-insectan metabolites of phytopathogenic fungi. These substances may be necessary for them to compete with phytophagous arthropods for a plant substrate. Extracts from the cultures of two wheat pathogens, *Parastagonospora nodorum* and *Bipolaris sorokiniana*, demonstrated aphidocidal activity, presumably due production of mycophenolic acid and sterigmatocystin, respectively. Monocerin, a

common metabolite of a number of phytopathogenic fungi from the genus *Exserohilum*, has insecticidal activity along with antifungal and phytotoxic properties. The toxin was found in another phytopathogenic fungus, *Macrophomina phaseolina*, which also produces other entomotoxic compounds such as mellein, cordycepin, and kojic acid (Gao et al., 2019). Recently, monocerin was isolated from the culture of the entomogenous fungus, *Setosphaeria rostrata*. An insecticidal tetramic acid derivative was found in the culture fluid of *Pyrenophora teres*, a leaf pathogen of barley. When added to artificial diet at a concentration of 100 µg/g, it inhibited the larvae growth in *Spodoptera exigua*, *Helicoverpa virescens*, *D. melanogaster*, and *Trichoplusia ni*. Diplosporin (a substance from the group of γ -pyrrolizidines) and chaetoglobosin K (from the group of cytochalasins) isolated from the culture of the corn stem rot pathogen *Stenocarpella maydis* showed moderate insecticidal activity against *S. frugiperda*: a decrease in the growth level by 50–75% at a concentration of 1 mg/g of artificial diet (Khambhati et al., 2020).

RNAi

RNAi is particularly efficient in some insects, especially those that belong to the order of Coleoptera, which respond acutely. Unfortunately, Lepidoptera and Hemiptera pests appear largely recalcitrant in their response to environmental RNAi, suggesting that there are biological barriers limiting RNAi use for management of these insects. Elucidating these bottlenecks is likely to allow this technology, with its unique and novel mode of action, to be integrated into Integrated pest management (IPM) strategies. The Canadian Food Inspection Agency (CFIA) approved in September 2016 the commercialization and release of a new maize event, MON87411, which has the commercial name SmartStax PRO, expressing three Crystal (Cry) genes and a dsRNA containing a 240-bp fragment of the *D. virgifera* Sucrose non-fermenting 7 (DvSnf7) gene. In June 2017, the United States Environmental Protection Agency (US-EPA) also approved this event for commercial planting. The DvSnf7 gene codes for an essential protein in vacuolar sorting and, until now, no insecticide has been developed that interferes with this process. However, as a consequence of the mechanism of RNAi, the Snf7 dsRNA alone takes a long time to effectively kill WCR. Therefore, the event MON87411 was developed in combination with the faster acting Cry genes from *Bacillus thuringiensis* (Bt) which will target both key lepidopteran pests and CRW as well as the *Diabrotica* spp. complex. The main purpose of the combination of these mechanisms (i.e. Bt and RNAi) is to reduce the occurrence of insects resistant to Bt technology. Expression of Snf7 dsRNA in transgenic maize has been demonstrated to protect maize roots from WCR larvae (Zotti et al., 2018).

While sprayable dsRNAs might be very effective against chewing insects feeding on the externally exposed parts of the plant, this is not the ideal approach for sap-feeding insects such as hemipterans. For these insects, a method called in plant system (iPS) was developed to target the Asian citrus psyllid (ACP), *Diaphorina citri*, using vegetative new growth flushes to deliver dsRNA. Using this method, transcript knockdown and psyllid mortality were achieved when ACP was fed with dsRNA targeting arginine kinase, a gene that plays a critical role in cellular energy and metabolism in invertebrates (Liu et al., 2019).

As a consequence of the massive use of pesticides, *T. urticae* has rapidly developed resistance to almost all types of acaricides. The availability of the *T. urticae* genome has contributed to understanding its post-transcriptional gene silencing mechanism, which has paved the way for the use of RNAi for mite control. The systemic/parental RNAi described above was used to suppress the expression of the Distal-less gene, a member of a homeotic gene family involved in appendage specification, for example limb morphogenesis. Adult females were injected with dsRNA or siRNA and produced offspring with truncated and fused leg segments. A protocol was established using leaf disc-mediated delivery of dsRNA through feeding. Using this protocol for silencing the coatamer subunit beta-2 gene which is responsible for the transport of biosynthetic membrane, resulted in the highest (>65%) mortality in *T. urticae*. This method could thus be applied for rapid screening of lethal genes. In another spider mite, *Panonychus citri*, which is a key pest of citrus crops and is resistant to most pesticides, RNAi was effective and gene silencing of Spook caused significant mortality of nymphs (56%) foraging on dsRNA-treated leaf discs. These results revealed that dsRNA targeting selected genes and delivered through feeding was able to induce gene knockdown and lethality in mites. Therefore, these target genes are useful candidates to be exploited for a mite control strategy (e.g. transgenic plants expressing dsRNA or sprayable dsRNA) in combination with other control strategies such as the use of Bt toxins and acaricides (Christiaens et al., 2021).

Certainly, insect resistance is always a concern when new control strategies are introduced. It is known that insects have great phenotypic and genetic plasticity, and some individuals in a corn rootworm population could be more or less sensitive to the DvSnf7 dsRNA trait introduced into the new maize event MON87411. An experiment using field-collected populations with and without crop rotation resistance demonstrated a differential response to ingested dsRNA treatments. This demonstrated that phenotypic responses to RNAi-based pesticides vary across corn rootworm populations and confirmed that there is a potential for resistance development. Following the principles of IPM would mitigate this selection in order to delay the onset of resistance. The possible mechanisms of resistance to transgenic RNAi crops discussed at a US-EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) meeting are as follows (Pan et al., 2016). Changes in dsRNA target sequence was considered improvable with a 240 bp target such as in the case of DvSnf7 dsRNA, but could occur with shorter targets, and the presence of single nucleotide polymorphisms could reduce the complementarity of the dsRNA with its target. Longer dsRNAs (>200 nt) may be advantageous as they result in a larger number of siRNAs matching the target mRNA and therefore could increase the RNAi response and prevent the selection of individuals as a result of the natural genetic variation. Changes in the kinetics of dsRNA imposed by natural barriers that are important for events such as uptake, (systemic) transport, among others Changes in the RNAi machinery enzymes or components such as reduction of recognition by the RISC complex of siRNA molecules, malfunction of the RISC complex in degrading the target mRNA, reduction in processing by Dicer ribonucleases, or the blocking of systemic spread of the RNAi signal. In addition, insects could develop different mechanisms to compensate for the specific gene silencing by increasing transcription rates or up-regulating other genes that can perform the same function as, or a similar function to, the silenced gene (Yoon et al., 2018). Also, resistance to dietary dsRNA could arise from reduced uptake during insect feeding on modified plants, which may present different olfactory or gustative cues. However, SmartStax PRO maize showed no difference from non-transgenic lines regarding these parameters. During the US-EPA FIFRA meeting, a report was presented showing that about 134 adults of corn rootworm emerged from crop plots treated with SmartStax PRO, but the attendees at the meeting stated that there was no evidence that those individuals were in fact resistant to dsRNA DvSnf7. A number of the mechanisms mentioned above could be potential resistance mechanisms, but to date none has been identified. Adoption of classical refuges, which are areas within the crop without the trait/pesticidal substance, and the combination of multiple insecticidal substances with

different modes of action as well as different control strategies delay the evolution of insect resistance to RNAi genetically engineered crops (Ren et al., 2019).

References

- Biedermann, P. H., & Rohlf, M. (2017). Evolutionary feedbacks between insect sociality and microbial management. *Current opinion in insect science*, 22, 92-100.
- Berestetskiy, A., & Hu, Q. (2021). The chemical ecology approach to reveal fungal metabolites for arthropod pest management. *Microorganisms*, 9(7), 1379.
- Butt, T. M., Coates, C. J., Dubovskiy, I. M., & Ratcliffe, N. A. (2016). Entomopathogenic fungi: new insights into host-pathogen interactions. *Advances in genetics*, 94, 307-364.
- Christiaens, O., Sweet, J., Dzhabazova, T., Urru, I., Smaghe, G., Kostov, K., & Arpaia, S. (2021). Implementation of RNAi-based arthropod pest control: Environmental risks, potential for resistance and regulatory considerations. *Journal of Pest Science*, 1-15.
- Gao, W.-B.; Liu, S.-S.; Tian, Y.-C.; Dong, Q.; Zhang, J.; Qin, J.-C.; Luo, D.-Q. Setosphaerine A: A New Chlorinated Polyketide Isolated From the Entomogenous Fungus *Setosphaeria rostrata*. *Nat. Prod. Comm.* 2019, 1–3.
- Gonçalves, F., Nunes, C., Carlos, C., López, Á., Oliveira, I., Crespi, A., ... & Torres, L. (2020). Do soil management practices affect the activity density, diversity, and stability of soil arthropods in vineyards?. *Agriculture, Ecosystems & Environment*, 294, 106863.
- Guedes, R. N. C., & Cutler, G. C. (2014). Insecticide-induced hormesis and arthropod pest management. *Pest Management Science*, 70(5), 690-697.
- Guedes, R. N. C., Smaghe, G., Stark, J. D., & Desneux, N. (2016). Pesticide-induced stress in arthropod pests for optimized integrated pest management programs.
- Khambhati, V.H.; Abbas, H.K.; Sulyok, M.; Tomaso-Peterson, M.; Shier, W.T. First Report of the Production of Mycotoxins and Other Secondary Metabolites by *Macrophomina phaseolina* (Tassi) Goid. Isolates from Soybeans (*Glycine max* L.) Symptomatic with Charcoal Rot Disease. *J. Fungi* 2020, 6, 332.
- Liu, X., Cooper, A. M., Yu, Z., Silver, K., Zhang, J., & Zhu, K. Y. (2019). Progress and prospects of arthropod chitin pathways and structures as targets for pest management. *Pesticide biochemistry and physiology*, 161, 33-46.
- Menta, C., & Remelli, S. (2020). Soil health and arthropods: From complex system to worthwhile investigation. *Insects*, 11(1), 54.
- Pan, H., Xu, L., Noland, J. E., Li, H., Siegfried, B. D., & Zhou, X. (2016). Assessment of potential risks of dietary RNAi to a soil micro-arthropod, *Sinella curviseta* Brook (Collembola: Entomobryidae). *Frontiers in plant science*, 7, 1028.
- Razzaq, Abdul, Arfan Ali, Muhammad Mubashar Zafar, Aisha Nawaz, Deng Xiaoying, Li Pengtao, Ge Qun et al. "Pyramiding of cry toxins and methanol producing genes to increase insect resistance in cotton." *GM Crops & Food* 12, no. 1 (2021): 382-395.
- Ren, Maozhi, Muhammad Mubashar Zafar, Huijuan Mo, Zhaoen Yang, and Fuguang Li. "Fighting against fall armyworm by using multiple genes pyramiding and silencing (MGPS) technology." *Sci China Life Sci* 62, no. 12 (2019): 1703-6.
- Roy, S., Roy, M. M., Jaiswal, A. K., & Baitha, A. (2018). Soil arthropods in maintaining soil health: thrust areas for sugarcane production systems. *Sugar Tech*, 20(4), 376-391.
- Safdar U, Ahmed W, Ahmed M, Hussain S, Fatima M, Tahir N, 2022. A review: pesticide application in agriculture and its environmental consequences. *Int J Agri Biosci*, 11(2): 125-130. <https://doi.org/10.47278/journal.ijab/2022.017>
- Saldaña, M. A., Hegde, S., & Hughes, G. L. (2017). Microbial control of arthropod-borne disease. *Memórias do Instituto Oswaldo Cruz*, 112, 81-93.
- Sanchez-Contreras, M., & Vlisidou, I. (2008). The diversity of insect-bacteria interactions and its applications for disease control. *Biotechnology and Genetic Engineering Reviews*, 25(1), 203-244.
- Yoon, J. S., Sahoo, D. K., Maiti, I. B., & Palli, S. R. (2018). Identification of target genes for RNAi-mediated control of the Twospotted Spider Mite. *Scientific Reports*, 8(1), 1-7.
- ZAFAR, Muhammad Mubashar, Abdul RAZZAQ, Muhammad Awais FAROOQ, Abdul REHMAN, Hina FIRDOUS, Amir SHAKEEL, Huijuan MO, and Maozhi REN. "Insect resistance management in *Bacillus thuringiensis* cotton by MGPS (multiple genes pyramiding and silencing)." *Journal of Cotton Research* 3, no. 1 (2020): 1-13.
- Zafar, Muhammad Mubashar, Ghulam Mustafa, Fiza Shoukat, Atif Idrees, Arfan Ali, Faiza Sharif, Amir Shakeel et al. "Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton." *Scientific Reports* 12, no. 1 (2022): 1-11.
- Zehnder, G., Gurr, G. M., Kühne, S., Wade, M. R., Wratten, S. D., & Wyss, E. (2007). Arthropod pest management in organic crops. *Annual review of Entomology*, 52, 57-80.
- Zotti, M., Dos Santos, E. A., Cagliari, D., Christiaens, O., Taning, C. N. T., & Smaghe, G. (2018). RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. *Pest management science*, 74(6), 1239-1250.