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## Advancements in Identifying Insect Resistance to Chemical Control

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### ABSTRACT

Increasing pesticide resistance has emerged as a major issue in both ecology and agriculture, complicating the control of pests and ectoparasites while exacerbating the environmental impact of chemical treatments. This paper highlights the importance of investigating insecticide resistance in the veterinary, agricultural, and medical contexts. It provides an overview of the resistance observed in ectoparasites and insect pests in different global regions against most used insecticides. The paper also describes key methods for detecting insecticide resistance in field populations. Furthermore, it summarizes recent advances in understanding the molecular mechanisms behind insecticide resistance, while identifying key research directions. The need to assess resistance profiles and monitor the potential for resistance development in field populations is emphasized. A deeper understanding of molecular mechanisms involved in resistance is crucial for the discovery of new insecticidal agents and the formulation of effective application strategies.

**Keywords:** Insecticide, Pests, Resistance profile, Resistance diagnostics, Molecular mechanisms

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### Introduction

The application of chemicals has long been the primary method for managing ectoparasites and insect pests [1, 2], and pesticides continue to be the most widely used tool for controlling their populations [3-5]. Over the past several decades, pesticide use in agriculture has risen significantly, driven by the growing demand for the production of global food [6, 7]. According to data from the Food and Agricultural Organization, pesticide consumption in 2019 reached 4.2 million tons, with insecticides accounting for 17% of the total, following herbicides (53%) and fungicides and bactericides (23%) [7]. In Russia, pesticide production has increased 1.7 times from 86.8 thousand tons in 2017 to 148.9 thousand tons in 2021, with insecticides making up 12.5% of the total by 2021 [8].

Insecticides are vital for boosting agricultural productivity [2, 7]. However, their excessive and prolonged use, coupled with higher application doses, frequent treatments, and the reproduction cycles of pests, creates a significant risk of developing pesticide resistance in insects and mites [9]. This resistance has become a critical challenge for both ecology and agriculture [10], as it complicates pest and ectoparasite management and contributes to a higher chemical burden on the environment [11].

This paper highlights the significance of investigating insecticide resistance in veterinary, agricultural, and medical contexts. It provides an overview of the resistance observed in ectoparasites and insect pests in various global regions against most used insecticides. The paper also outlines key methods for detecting insecticide

resistance in field populations. Furthermore, it summarizes recent advancements in understanding the molecular mechanisms behind insecticide resistance, while identifying key research directions.

## Results and Discussion

Research on insecticide resistance has grown significantly over the years, focusing on its prevalence in insect populations and the mechanisms responsible for its development. The increasing number of publications on this subject highlights its relevance, with indexed articles in Scopus nearly doubling from 616-1302 between 2011 and 2021, and maintaining this trend over the past 5 years. Now, over 330 insecticides are known to exhibit resistance in various arthropod species [12]. Bass *et al.* [13] conducted an analysis of neonicotinoid resistance and its forming mechanisms in pest populations that include cotton whitefly, green peach aphid, cotton aphid, brown planthopper, and Colorado beetle, all of which have significant economic importance. Resistance patterns in diamondback moths (*Plutella xylostella*), a common cruciferous pest, to OPCs, pyrethroids, and biopesticides have also been reported across various geographic regions [14, 15]. Between 2019 and 2020, high resistance to OPCs and growth regulators, along with tolerance to neonicotinoids, was found in populations of the rice pest white-backed planthopper (*Sogatella furcifera*) in China [16]. In African countries, Van den Berg *et al.* [17] noted the increasing risk of insecticide resistance in corn, cotton, vigna, and tomato pest species. Research conducted in Russia has also identified resistance in several insect populations, including the Colorado beetle, which has developed increasing resistance to various insecticides in areas such as the North Caucasus, Central Russia, and the North-Western regions [18]. Similar resistance trends have been observed in green peach aphids in the Astrakhan region and foxglove aphids in the Leningrad region, as well as in wireworms in the Pskov region [18].

The challenge of insecticide resistance extends beyond agriculture to impact both animal health and medicine [19-26]. Evidence suggests that cross-resistance is common in mosquito populations with public health relevance. For instance, between 2000 and 2020, resistance was reported in mosquitoes from the genera *Anopheles*, *Aedes*, *Culex*, and *Culiseta* in Iran, where these populations showed resistance to a variety of insecticides, including OPCs, COCs, pyrethroids, and carbamates [27]. During the same period, South Asian countries observed a rise in insecticide resistance within *Aedes* mosquito populations, which are vectors for diseases like Dengue, Zika, and yellow fever [28, 29]. Additionally, bed bugs (*Cimex lectularius* L.) have shown resistance to pyrethroids, neonicotinoids, and fipronil [24]. Juache-Villagrana *et al.* [30] discussed how insecticide resistance affects the vector competence of arthropods that transmit infections [30]. In Ireland, red lice (*Bovicola bovis*) collected from livestock demonstrated tolerance to deltamethrin, a pyrethroid insecticide [31]. The global challenge of insecticide resistance is also seen in housefly populations (*Musca domestica* L.) [32]. Resistance in houseflies to major classes of insecticides, such as organophosphates (OPCs) and carbamates, has been documented across America, Africa, and Asia [33, 34]. Populations resistant to pyrethroids and neonicotinoids have been reported in flies on farms with livestock and poultry [35-37]. Resistance to several insecticide classes, including COCs, OPCs, carbamates, pyrethroids, and neonicotinoids, has also been observed in housefly populations in Russia [32].

Several studies suggest the need for early identification of insecticide resistance, along with continuous monitoring of the spread in insect populations and effective resistance management strategies [11, 17, 20, 24, 38]. The World Health Organization (WHO) reports that only 38% of European countries factor in insecticide susceptibility levels when selecting insecticides for controlling insect vector populations. In contrast, in regions such as Asia-Pacific, Africa, and South America, the percentage of countries employing this practice is significantly higher, ranging from 80-92% [39]. Gaining insight into the resistance profiles of insect populations not only makes pest and parasite control more efficient and cost-effective but also aids in minimizing chemical use and reducing environmental harm.

To prevent and address insecticide resistance in pest and parasitic insect species, it is critical to gather detailed resistance profile data from natural populations. This is achieved through toxicological and molecular-genetic approaches. Toxicological testing involves biotesting to measure the toxicity of insecticides, resulting in the calculation of the resistance ratio (RR) and the proportion of individuals susceptible to a given insecticide at a diagnostic dose. The resistance ratio is determined by comparing the median lethal concentration (LC<sub>50</sub>) of the insecticide for the target population to the LC<sub>50</sub> value for a susceptible strain. WHO advocates using diagnostic doses or concentrations to quickly assess resistance, which is defined as those causing 95-99% mortality in a susceptible insect population [26].

Specialists determine specific diagnostic concentrations (or doses) for every insect to assess their susceptibility to insecticides. A population is considered susceptible if 98-100% mortality occurs at the diagnostic concentration, whereas a population is deemed resistant if less than 90% mortality is observed at the same concentration [26]. The methods used to evaluate insecticide toxicity are tailored to the biological traits of the insect species or groups under study. The WHO has developed procedures to identify insecticide resistance in populations of disease-carrying vectors and other synanthropic insects. Further details on testing methods for plant pests can be seen on the Insecticide Resistance Action Committee's website.

Following the WHO diagnostic protocols, it is not enough to merely confirm the presence of resistance in an insect population. It is also crucial to assess the extent of resistance and identify the underlying mechanisms [26]. This involves using biochemical and molecular techniques to pinpoint the mechanisms responsible for insecticide resistance in specific populations. Studies focus on detoxifying enzymes [40, 41], insecticide molecular targets [3, 42], and the identification of alleles linked to resistance [43, 44]. Recent advancements have been made in the knowledge of the mechanisms of resistance to widely used insecticides, including pyrethroids, OPCs, neonicotinoids, spinosyns, and pyrazoles. For example, five alleles responsible for resistance to pyrethroids due to target site insensitivity have been identified in the literature: *kdr-his* (L1014H), *kdr* (L1014F), *super-kdr* (M929I+L1014F), *Type N* (D600N+M918T+L1014F), and *1B* (T929I+L1014F) [44]. Mutations such as Gly137Asp and Trp251Leu/Ser in carboxylesterase genes are known to confer resistance to OPCs by altering the enzyme's active site and enhancing its hydrolytic activity against the insecticide [45]. Carboxylesterases in the cotton bollworm *Helicoverpa armigera* Hbn. contribute to resistance against organophosphates and pyrethroids by increasing sequestration through gene overexpression [46]. Feyereisen *et al.* [47] reviewed mutations in acetylcholinesterase genes that contribute to OPC resistance in various insect species. While resistance mechanisms to newer insecticides like chlorfenapyr and chlorantraniliprole remain less clear, possible resistance mechanisms for chlorfenapyr have been explained in detail for the spider mite [48] and boll weevil [49], including increased activity of esterases and glutathione-S-transferases, along with reduced cuticle permeability.

Research on resistant populations of cabbage moths has shown that the enzymes previously discussed are not considered in developing resistance to chlorfenapyr [50]. A significant focus of insecticide resistance studies is on epigenetic influences, the interactions between resistance-related genes, and the regulatory factors that activate their expression [38]. Categorizing and re-defining particular genetic mutations linked to resistance against specific insecticides could be valuable for creating molecular diagnostic tools for detecting resistance [38]. Furthermore, understanding the molecular mechanisms behind resistance to various insecticides, as well as its potential emergence in insect populations, is crucial for discovering new active ingredients and formulating innovative pesticides, as well as devising new strategies for their use.

## Conclusion

In conclusion, the cases discussed in this review highlight that insecticide resistance is a critical concern for both ecology and agriculture. By conducting toxicological and molecular research on the resistance profiles of insect populations, more effective resistance management strategies can be developed, ultimately leading to a reduction in the chemical burden on the environment.

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