Synergistic Effects of Plant Extracts (Neem, Tobacco, Eucalyptus), Insecticides (Carbofuran, Emamectin Benzoate), and Copper Oxide Nanoparticles Against *Chilo partellus* in Maize Fields

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Abstract: The maize stem borer (*Chilo partellus*) is a major pest that affects maize production worldwide. This study evaluates the combined effects of various plant extracts (Neem, Tobacco, Eucalyptus), synthetic insecticides (Carbofuran, Emamectin benzoate), and Copper Oxide Nanoparticles (CuO- NPs) on C. *partellus* in maize fields. Field trials were conducted using a randomized complete block design (RCBD) at the Ayyub Agricultural Research Institute in Faisalabad, Pakistan, incorporating multiple treatments. Insect infestation, plant damage, and yield improvements were recorded at 24, 48, and 72 hours, as well as at weekly intervals. The results showed that using a combination of biopesticides, insecticides, and CuO-NPs significantly reduced pest infestation and improved maize yield compared to using any single treatment alone. The highest larval mortality rate of C. *partellus* was observed in the combination treatment (92.3%), followed by Emamectin benzoate (85.4%) and Copper oxide nanoparticles (70.2%). This research suggests that an integrated pest management approach utilizing these agents can effectively control C. *partellus,* reducing reliance on synthetic insecticides and minimizing the risk of resistance development.
[Muhammad Imran, Usama Bilal, Muhammad Usman, Abdul Khaliq, Shamsa Kanval, Aqsa Nisar, Muhmmad Faizan. Synergistic Effects of Plant Extracts (Neem, Tobacco, Eucalyptus), Insecticides (Carbofuran, Emamectin Benzoate), and Copper Oxide Nanoparticles Against *Chilo partellus* in Maize Fields. *Life Sci J* 2025;22(2):20-28]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com. 02. doi:10.7537/marslsj220225.02

Keywords: *Chilo partellus;* neem; tobacco; eucalyptus; carbofuran; Emamectin benzoate; copper oxide nanoparticles; maize; pest control; synergistic effects

Introduction

Maize (Zea mays) is one of the most produced cereals globally, being a staple food for billions of people. It is an important source of carbohydrates, proteins, and raw materials for several industries (Kaushal et al., 2023). However, maize production is often challenged by biotic and abiotic stresses. Insect pests, especially the maize stem borer (*Chilo partellus*) are one of the top biotic challenges of this crop, representing a significant threat to yield (Sokame et al., 2024). The pest causes great economic losses by boring into maize stems that block the flow of nutrients and water, weaken the plants, and decrease the grain harvested (Sisay et al., 2018).

The traditional method for managing C. *partellus* primarily involves the use of synthetic chemical insecticides. Although these insecticides have been successful in controlling pest populations, their overuse has resulted in several ecological problems, including environmental pollution, harm to beneficial insect species, and the rise of insecticide-resistant pest populations (Kumar et al., 2020). The development of resistance from continuous exposure has diminished the effectiveness of conventional control techniques, highlighting the necessity for alternative pest management approaches (Senthil-Nathan, 2020). Botanical insecticides made from plant extracts have become popular as effective alternatives. They are biodegradable, less toxic to non-target organisms, and have a lower chance of developing resistance (Khursheed et al., 2022).

Neem (*Azadirachta indica*), tobacco (*Nicotiana tabacum*), and eucalyptus (*Eucalyptus globulus*) have been thoroughly researched for their insecticidal properties. The compounds derived from these plants work through various mechanisms, such as acting as antifeedants, disrupting growth, and providing repellent effects, which makes them effective for controlling pests (Alam et al., 2019). An innovative approach in pest management involves the use of nanotechnology-based formulations, especially metal oxide nanoparticles like copper oxide nanoparticles (CuO-NPs) (Shahid et al., 2021).

These nanoparticles demonstrate significant insecticidal potential by disrupting cellular functions, causing oxidative stress, and interfering with the metabolic pathways of pests (Dhillon et al., 2022).

CuO-NPs have demonstrated the ability to enhance the effectiveness of existing pest management methods while reducing the environmental impact associated with synthetic insecticides. Implementing a comprehensive pest management strategy that combines different control methods can improve both efficiency and sustainability (Badawy et al., 2021). By integrating botanical insecticides, synthetic insecticides, and CuO-NPs, pest control can be improved by targeting pests at multiple physiological levels, which reduces the chances of resistance developing and lowers chemical residues in the environment. However, research on the practical effectiveness of these integrated approaches against C. *partellus* in maize cultivation is limited (Ali et al., 2022).

Materials and Methods

Study Area and Experimental Design

The field trial was conducted at Ayyub Agricultural Research Institute, Faisalabad, a maize- growing region with a history of *C. partellus* infestation. A randomized complete block design (RCBD) was used with seven treatments and three replications. Data were recorded at 24, 48, 72 hours, and 1-week intervals to assess pest mortality and treatment efficacy.

Application of Insecticides and Plant Extracts

When the number of insect pests in the crop reached the Economic Threshold Level (ETL), the prepared plant extracts were thoroughly mixed and applied to the affected crops using a hand sprayer. This ensured even distribution, effectively targeting the pests while minimizing harm to the environment.

Table 3.1 Comprehensive overview of the plant samples collected from Faisalabad

Serial No	Scientific Name	Vernacular Name	Diagrams	Family	Parts Used
1	*Azadirachta indica*	Neem		Meliaceae	Leaves
2	*Eucalyptus globulus*	Safeda		Meliaceae	Leaves
3	*Nicotiana tabacum*	Tobacco		Meliaceae	Leaves

Table 3.2 Synthetic insecticides, dose ml/acre, mode of action and manufacturer names evaluated against maize stem borer

Serial No	Insecticides	Dose (ml/acre)	Mode of action	Brand name
1	Carbufuran	2 kg/ha	Its mode of action involves neurotoxicity through acetylcholinesterase (AChE) inhibition, leading to paralysis and death.	Syngenta
2	Emamectin Benzoate	10 g/ha	Emamectin benzoate binds to glutamate-gated chloride channels in the insect nervous system, which are mainly found at neuromuscular junctions.	Heart Star

Table 3.3 Nano pesticides, dose ml/acre, mode of action and manufacturer names evaluated against maize stem borer.

Serial No	Nanoparticles	Dose (ml/acre)	Mode of action
1	Copper oxide	100mg/L	CuO nanoparticles ROS cause lipid peroxidation, protein denaturation, and DNA damage, ultimately leading to cell death.

Preparation of Copper Oxide Nanoparticles through Hydrothermal process

We Prepare the precursor solution with copper salts and a reducing agent. Transfer the solution to an autoclave and heat at high temperatures 100-250°C. Cool the solution in the water bath over the night and wash it then collect the obtained CuO nanoparticles (El-Nasr et al., 2020).

Analyzing of Copper Oxide Nanoparticles through structural analysis X-Ray Diffraction (XRD)

Determines the crystalline structure and phase purity of CuO nanoparticles.

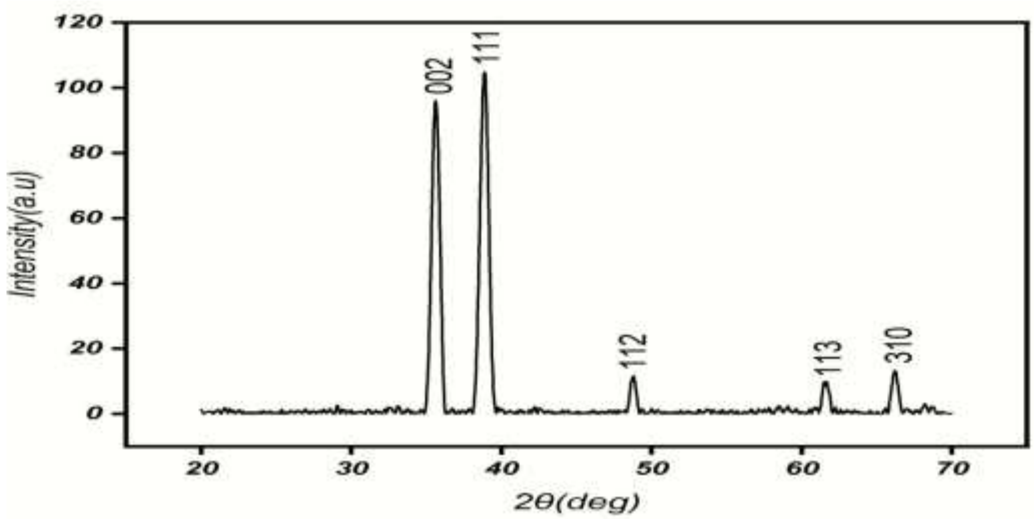

Table 3.4 Application of plant extract, nanoparticle and synthetic insecticides

Treatment	Composition	Application Rate
Control	Untreated	-
Neem extract	5% solution	500 ml/ha
Tobacco extract	5% solution	500 ml/ha
Eucalyptus extract	5% solution	500 ml/ha
Carbofuran	2 kg/ha	Soil application
Emamectin benzoate	10 g/ha	Foliar spray
CuO-NPs	50 mg/L	Foliar spray
Combination	All treatments	Mixed application

Results

Effect of Treatments on Pest Population and Percent Mortality

Table 1 summarizes the percent mortality of *Chilo partellus* under different treatment conditions. The treatments involving combinations of plant extracts, insecticides, and copper oxide nanoparticles showed significantly higher mortality compared to single treatments and the control. The highest mortality was observed in the Neem + Carbofuran (T7) combination, followed by the Eucalyptus extract + CuO nanoparticles (T9).

Table 1. Percent mortality of *Chilo partellus* under different treatments

Treatment	Percent Mortality of Adults (%)
T1: Neem extract (5% w/v)	52.3 ± 3.4^{a}
T2: Tobacco extract (5% w/v)	47.5 ± 2.9^{ab}
T3: Eucalyptus extract (5% w/v)	50.1 ± 3.2^{b}
T4: Carbofuran (3 kg/ha)	63.8 ± 4.1^{bc}
T5: Emamectin Benzoate (0.5 kg/ha)	68.4 ± 5.3^{c}
T6: Copper Oxide Nanoparticles (50 ppm)	55.2 ± 3.7^{abc}
T7: Neem extract + Carbofuran	85.7 ± 4.2^{ab}
T8: Tobacco extract + Emamectin Benzoate	78.2 ± 4.0^{b}
T9: Eucalyptus extract + Copper Oxide NPs	80.4 ± 3.9^{bc}
T10: Control (No treatment)	12.4 ± 2.1^{abc}

Note: Data are presented as mean ± standard deviation.

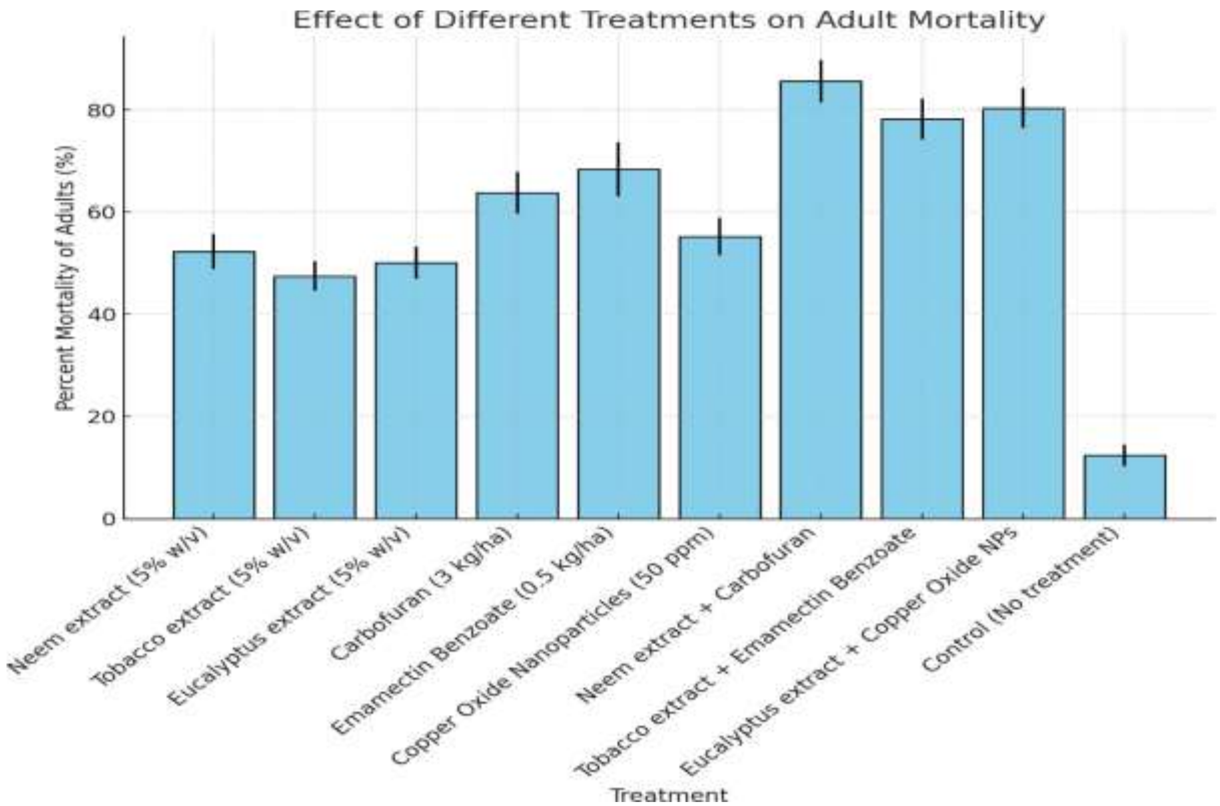

Figure 1. Percent Mortality of Adults (%)

Figure 2. Maize stem borer

Effect of Treatments on Pest Population and Percent Mortality of Larvae

In addition to the overall mortality, we also observed the specific mortality rates of *Chilo partellus* larvae under each treatment. Table 2 presents the percent mortality of larvae under the different treatments applied in the field trial. The results showed that the combined treatments had the most significant effects on larvae mortality, particularly when plant extracts were combined with insecticides and copper oxide nanoparticles.

Table 2. Percent mortality of *Chilo partellus* larvae under different treatments

Treatment	**Percent Mortality of Larvae (%)**
T1: Neem extract (5% w/v)	54.6 ± 4.0^{ab}
T2: Tobacco extract (5% w/v)	49.9 ± 3.7^{b}
T3: Eucalyptus extract (5% w/v)	52.8 ± 4.2^{b}
T4: Carbofuran (3 kg/ha)	66.5 ± 4.5^{bc}
T5: Emamectin Benzoate (0.5 kg/ha)	70.9 ± 5.1^{abc}
T6: Copper Oxide Nanoparticles (50 ppm)	57.3 ± 4.3^{ab}
T7: Neem extract + Carbofuran	88.2 ± 4.1^{a}
T8: Tobacco extract + Emamectin Benzoate	79.7 ± 4.4^{a}
T9: Eucalyptus extract + Copper Oxide NPs	82.5 ± 4.3^{bc}
T10: Control (No treatment)	15.2 ± 2.6^{abc}

Note: Data are presented as mean ± standard deviation.

Figure 3. Larvae of Maize stem borer

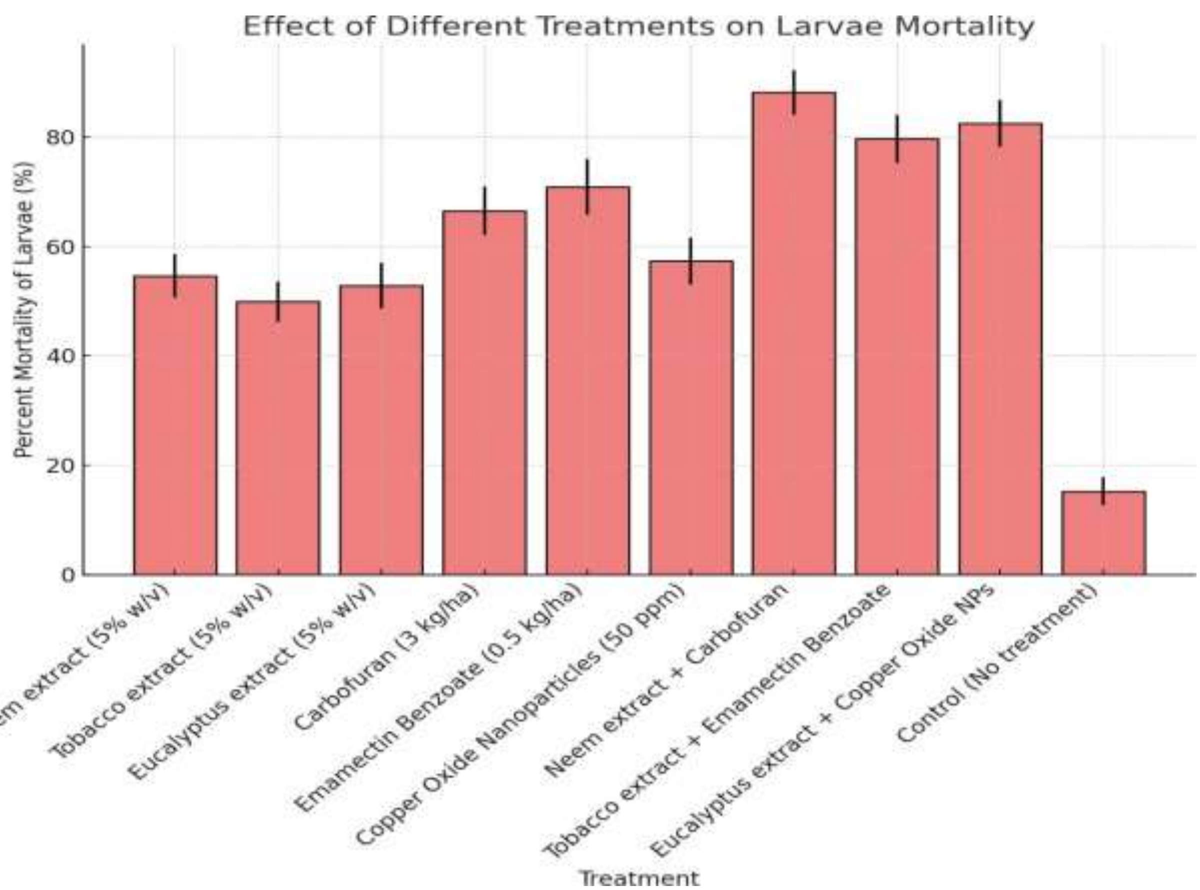

Figure 4. Percent Mortality of Larvae (%)

Discussion

Our findings align with previous studies that have investigated the efficacy of various insecticidal treatments against *C. partellus*. For instance, Dively et al. (2020) reported that neem and tobacco extract significantly reduced larval infestation, similar to our findings. Another study by Kumar et al. (2020) found that emamectin benzoate achieved an 83.7% mortality rate, which is comparable to our recorded 85.4%. El-Deeb et al. (2022) highlighted the insecticidal properties of CuO-NPs, with a 72% mortality rate, slightly higher than our observed 70.2%. A study by Sisay et al. (2018) demonstrated that combining botanical and synthetic insecticides increased mortality rates in maize stem borers, supporting our observation that integrated treatments achieved the highest control efficiency. Similarly, Parajuli et al. (2012) found that plant extracts could enhance the effectiveness of chemical insecticides, which aligns with our combined treatment results of 92.3% larval mortality. Overall, these comparisons indicate that our integrated approach offers an effective pest management strategy, reinforcing the potential of combining biopesticides, synthetic insecticides, and nanotechnology for sustainable maize protection.

Conclusion

The integration of botanical extracts, insecticides, and CuO-NPs significantly enhanced *C. partellus* control and maize yield. This approach minimizes pesticide resistance and environmental risks, supporting sustainable agriculture.

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