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Biochar Reduces the Pupation and Eclosion of a Specialist (Manduca sexta) and a Generalist Pest (Spodoptera frugiperda)

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BIOCHAR REDUCES THE PUPATION AND ECLOSION OF A SPECIALIST (*MANDUCA SEXTA*) AND A GENERALIST PEST (*SPODOPTERA FRUGIPERDA*)

A Thesis

by

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ABSTRACT

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Chapter 1: This chapter incorporates detailed information about the biochar concept, its production, composition, and properties, harmful arthropods and their damage, the effect of soil on the arthropod community, and the application of biochar on arthropod control.

. Chapter 2: The comprehensive lab experiment was conducted with modified pupation media enriched with walnut shell biochar (Chapter II) to evaluate the effects of biochar on the pupation and eclosion success rates of a specialist (*Manduca sexta*) and a generalist pest (*Spodoptera frugiperda*). We found that the biochar treatment led to a significant reduction in pupal mass, adult mass, adult wingspan, body length, and length of survival compared to the control. This was vital to understanding the direct impact of biochar on pupation and eclosion stages of pest development and serves as the foundation for future research of biochar on the soil to assess the effect of biochar on different stages of herbivores.

Chapter 3: This chapter provides an overview of my major findings and possible future directions for research to understand the biochar effect on arthropod's control and potential solutions to incorporate different types of biochar on herbivores' control and suggesting the biochar as a potential alternative against chemical insecticides for future references.

DEDICATION

I would like to dedicate my thesis to my mother, Karuna Khanal Wagle, and father, Ramesh Prasad Wagle, alongside my brother Niswarth Wagle, my grandfather Kalika Prasad Wagle, and my grandmother Devi Wagle for supporting me and providing unconditional love throughout my life.

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CHAPTER I

LITERATURE REVIEW ON THE EFFECT OF BIOCHAR ON BENEFICIAL AND HARMFUL ARTHROPODS

Abstract

It is undeniable that harmful arthropods cause significant loss to plant productivity in many ways in natural settings and agroecosystems. Many integrated pest management practices have been practiced throughout the years to reduce the severity of insect pest infestation and promote eco-friendly management techniques. Interestingly, only a few studies have examined the potential of biochar as an alternative pest management strategy that entertains anti-herbivore defense, affecting herbivores' survival and performance. Biochar is a solid stable charcoal-like substance made by the pyrolysis of organic material from agricultural and forestry waste. While it is beyond dispute that biochar is well documented for increasing crop productivity and altering soil physicochemical conditions underlying several environmental and agricultural benefits, it also provides an opportunity to control arthropod pests. The biochar effect has been primarily studied in different life stages of herbivores with different modes of infestation on plants. However, at present, we lack an updated review of their functionality in insect-plant interactions, especially focusing on their insect deterrent and/or insecticidal activity. In this review, we try to focus on the role of biochar in plant-insect interactions and plant defenses when soil is amended with biochar including biochar's structure, production, composition, regulation, and insecticidal as well as toxicity effects. Details on mechanisms underlining the interactions and effects of biochar are discussed in the context of insect-plant interactions, current studies, and potential recommendations for future research in this area.

Background

After the green revolution during the 1960s, agriculture has undergone a technological revolution, which drastically altered farming operations. The agriculture system at present has a major challenge to reconcile the flourishing demand for food with more sustainable agriculture practices (Calicioglu et al., 2019).

It has been postulated that by 2050, there will be 9.4 to 10.1 billion people on the planet, and by 2100, there will be an additional 2.7 billion people (FAO, 2015; UNDESA, 2017; United Nations, 2019). The global population is expected to reach 9.7 billion by 2064, according to the most recent estimates, and by 2100 it will have decreased to 8 billion (Vollset et al., 2020). Also, (Alexandratos and Bruinsma 2012) predicted that the world's demand for food will rise by 60% between 2005/2007 (the base period) and 2050, and that global crop demand may rise by 100% to 110% within the same time frame (Tilman et al., 2011). In many parts of the world, the problem is compounded by the fact that hunger and malnutrition are so pervasive and long-lasting. As things are, we won't be able to end world hunger by 2030 and before 2050 at the earliest (FAO, 2017). Besides a fundamental role in promoting global food security and eradicating worldwide famine and hunger, agricultural productivity plays a crucial role in promoting sound health, reducing rural poverty, promoting employment opportunities, and fostering biodiversity (Uphaus, 2008; Viana et al., 2022). To fulfill these major benefits, and meet the future demand of the expanding population, globally and particularly, regional agricultural productivity must be increased, but sustainably without deteriorating the environment. There are, however, many issues at present that have hampered agricultural productivity.

One of the issues faced by agricultural productivity at present is intensive agriculture. Intensive agriculture evolved as a need to feed the large population of human beings. It has served the purpose of producing a large quantity of food with limited resources to feed human beings, but it is not free from side effects as 94.8% of food provided to human beings throughout the year uses a high-energy input system, utilizing 30-50% more energy compared to the organic system (Ziesemer, 2007), and causes 61.7% destruction to soil organic matter (Kughur et al., 2015).

Second-generation issues have arisen because of agricultural intensification driven by the reckless and relentless use of chemical fertilizers and pesticides. These include the pollution of water bodies and the degradation of soils, the extinction of beneficial plants, insects, and other wildlife, and the poisoning of farm workers due to toxicity (Hans and Farooq, 2000; Kughur, 2012; Hu et al., 2015). Intensive farming can have a severe impact on soil such as acidification, nitrification, desertification, a decline in soil organic matter, soil contamination (e.g., by heavy metals and agrochemicals), soil compaction, and erosion (Zhou et al., 2021). This leads to less diversified soil food webs and fewer functioning groupings of soil biota, according to one research (Onder et al., 2011; Lazcano et al., 2021). Agricultural output may be severely hampered if soil biodiversity were to decline because of intensified land usage (Tsiafouli et al., 2015). Furthermore, agricultural intensification has caused significant reductions in pollinator biodiversity globally (Millard et al., 2021). The indiscriminate use of pesticides can also have a significant economic impact by lowering or offsetting the costs associated with plant diseases, insect pests, and weeds. Furthermore, rising residues of agricultural chemicals in soil, water, air, agricultural products, and even human blood and adipose tissue has been reported by many nations, despite the fertilizer application playing an important role in providing a variety of nutrients required for the growth of crops and an increase in production yield (Li et al., 2021; Iqbal et al., 2020).

Considering relentless reliance on chemical fertilizers, and pieces of machinery that emit GHGs and carbon dioxide into the atmosphere which degrade the soil, water, and ecosystem, this system is not built to last, and new agriculture practices i.e., organic farming, agroforestry, cover cropping, crop rotation should be entertained to overcome these prevalent problems by enhancing crop productivity and by long-term carbon sequestration (Nakka, 2016; Matuštík et al., 2020). Hence, a new approach to the diversification of agriculture and sustainable agriculture practices is a must to ensure global agriculture productivity.

Sustainable agriculture is important to improve food security and improve the environmental friendliness of various agricultural activities and performance (Nowak et al., 2019; Sharma et al., 2020). There have been several attempts to link sustainable agricultural development to agriculture, including biological farming (Mzoughi, 2011), ecological farming (C osta, 2010), etc.

With respect to that, different methods of sustainable agriculture such as permaculture, biodynamic framing, urban agriculture, hydroponics, agro-forestry, polyculture, crop rotation, natural animal raising, mulching, ground covers, and Integrated Pest Management (IPM) are being increasingly popular and widely adopted by farmers throughout the globe (Das et al., 2020). These sustainable agriculture practices provide greater efficiency in the use of natural resources (Francis and Porter, 2011), increased soil fertility (Leite et al., 2014), biological control of insect pests (Altieri et al., 2017), less soil, air, and water pollution (Leite et al., 2014).

Biochar: a sustainable farming strategy with great potential

Biochar, among the many conservation agricultural practices, has the potential to prove itself as a fundamental and easily accessible input for sustainable agriculture by reducing the use of scarce water by conserving water resources, improving soil fertility to effectively trap a substantial quantity of carbon in soil over time, increasing crop production, preserving ecosystem health, and decreasing greenhouse gas emissions (Semida et al., 2019; Smith et al., 2019). Therefore, biochar lessens the need for fertilizers, maintains soil health, offers the chance to aid agricultural lands in recovering from climate change-related extreme weather events, and promotes food security. Biochar has thus become even more pivotal in this recent era, taking into consideration the mess and hazard the modern practices have created.

Biochar is a solid stable carbon-rich compound that is formed by the pyrolysis of biomass and organic wastes such as manure, litter, wood chips, husks, peels, seeds, straw, and sewage sludge in the oxygen-limited environment and at temperatures of 250-800°C (Chan et al., 2007;Wang et al., 2019). Biochar is often referred to it as "agrichar", or charcoal for agricultural use as charcoal and biochar look similar to each other and share the same chemical properties (Lehmann and Joseph, 2015). Charcoal is used solely as a fuel in the combustion process, but biochar has a wide variety of other purposes (Godlewska et al.,2021).

Biochar Production

Biochar is made by different types of methods. One of the common techniques of biochar production is thermochemical conversion. The thermochemical conversion method consists of pyrolysis, carbonization, gasification, and torrefaction (Lin et al., 2016; Pang, 2019). Pyrolysis is the thermal breakdown of organic compounds at temperatures between 300 and 900 degrees Celsius in the absence of oxygen (Osayi et al., 2014). It converts waste biomass such as feedstock residues, manure, or woody materials into value-added products like biochar, syngas, and bio-oil. During the process, lignocellulosic components like cellulose, hemicellulose, and lignin undergo processes such as depolymerization, fragmentation, and cross-linking at specific temperatures which results in a different state of products like solid, liquid, and gas (Lappas et al., 2002). Hydrothermal carbonization is another cost-effective method of biochar production which is performed at a low temperature of around 180-250 °C (Lee et al., 2018). The product obtained from the hydrothermal process is known as hydrochar to differentiate the product produced from dry processes like pyrolysis and gasification (Fang et al., 2018). Gasification is a thermochemical method of decomposition of the carbonaceous material into gaseous products i.e., syngas consisting of CO, CO_2, CH_4, H_2, and traces of hydrocarbons in presence of agents such as oxygen, air, steam, etc and high temperature (Cha et al., 2016; You et al., 2017). The primary component that determines the amount of syngas produced is the Reaction Temperature. It was found that as temperature increased carbon monoxide and hydrogen production increased while other contents such as methane, carbon dioxide, and hydrocarbons decreased (Prabakar et al., 2018). Torrefaction is a recently developed technique for biochar production. It uses a low heating rate, earning the name mild pyrolysis (Gan et al., 2018). Various decomposition procedures remove the oxygen, moisture, and carbon dioxide from the biomass using inert atmospheric air in the absence of oxygen at a temperature of 300 degrees Celsius. It is best used for producing algae-based biochar (Yu et al., 2017). Biochar can be produced locally without much effort and costs. (Owsianiak et al., 2021) in his study found that currently six developing and middle-income countries, like Indonesia, Ethiopia, Kenya, Peru, Vietnam, and China are using low-cost simple kilns to manufacture biochar and exploiting it in the agricultural and industrial fields to reap benefits from it.

Composition and properties of biochar

Structurally, the main element present in the structure of the biochar is carbon (C), constituting more than 65% while other elements are hydrogen (H), oxygen (O) and small amounts of nitrogen (N) and sulfur (S) (Liu et al., 2015; Wijitkosum & Jiwonok, 2019). Furthermore, in biochar 12 carbon contain layers of graphene and graphene oxide (GO) whose edge carbons are reactive 13 due to presence of -COOH; -OH and -O- functional group that binds CO_2 on its sites and provide biochar with capacity of carbon sequestration (Chen et al., 2014). The biochar's molecular structure reflects the high degree of chemical and microbial stability (Lehmann et al., 2011).

Biochar has its own set of properties such as large specific surface area, high mineral content, and surface functional groups that affect physiochemical properties such as pH, porosity, and volatile matter content, dissolved organic matter, ash, and carbon. These properties, in turn, depends upon the type of organic input material present in biochar, the residence time of volatiles and the temperature during pyrolysis (Keiluweit et al. 2010; Hartley et al., 2016; Tomczyk et al., 2020). For instance, the temperature of pyrolysis affects the surface area and structure of the biochar (Wang et al., 2020). Thus, temperature and organic matter should be carefully selected to increase the effectiveness and productivity of biochar.

Harmful Arthropods and damage

Every organism has their own ecological role and might not directly benefit the plant we raise for consumption or economy. Even insects that we termed as harmful might help plants grow by aerating the soil and improving the water retaining capacity of soil (Faqir et al., 2021; Yoon et al., 2019). While it is true, there are some arthropods that can cause harm to plants. It

can be difficult to classify arthropods as 'harmful' and 'beneficial' as some might indirectly affect or harm the plant while even within the same family, one class may benefit the plant while some might not. Insects and mites together can cause significant damage to plants. In insects, order Orthoptera, Hemiptera, Thysanoptera, Coleoptera, Lepidoptera, and Diptera are considered the most destructive to agricultural crop (Triplehorn and Johnson 2005);
whereas Trombidiformes, Sarcoptiformes order are invasive mites order (Krantz and Walter, 2009).

The damage by arthropods to a crop can be of different types: direct and indirect damage. The direct damage can be a result of insects that directly feed that plant, especially the leaves. Within this type falls orthopterans, hemipterans, lepidopterans, etc. An arthropod can indirectly damage a plant by acting as a vector and carrying certain diseases. *Ricania simulans,* a hemipteran, is a very prominent polyphagous pest and sucks sap from plants causing harm to it. In regions where chemical use is prohibited, scientists are constantly seeking a way to control this pest naturally using various molecular editing and biotechnological methods (Gokturk et al., 2018). Aphids, because they directly feed on plants and spread numerous plant viruses, are widespread throughout the world and are regarded as serious agricultural pests. Despite enhanced pesticide use, foliage and root feeding herbivores are found to remove more than 20% of net plant productivity at all vegetation systems (Agrawal, 2011). (Culliney, 2014) reported an estimated 18-26% of global crop destruction annually.

Insect pests is found to suppress photosynthesis too by defoliating leaves leading to severed vasculature and defense-induced autotoxicity. For example, Zangerl et al., 2002 reported that caterpillars reduced photosynthesis by 20% when only 5% of area an individual wild parnship leaf was defoliated. (Heng-Moss et al., 2006) postulated that insects attack on specialized tissues, xylem and phloem causes more considerable loss in photosynthetic capacity.

Aphid outbreaks that result in significant crop losses are frequently reported because of the complexity of plant-aphid interactions and the quick emergence of pest biotypes that are resistant to them (Smith & Chuang, 2014; Yu et al., 2014). Losses of between $1 and $4 billion dollars' worth of crops annually have been attributed to insect damage to maize plants in 12 major maize-growing nations (Silver, 2019). In Africa alone, bug infestation occurred in 2016, and caused more than 50% yield loss of maize. Similarly, in India, maize stalk borer, *C. partellus* solely causes 26.7–80.4% yield loss among different agroclimatic regions (Reddy and Zehr, 2004). In Czech Republic, insect pests caused 16% total damage to sunflower seeds (Skoták et al; 2022). In Aligarh part of India, (Ahmed et al., 2009) found that infestation of Plutella xylostella (Linnaeus) in Cauliflower increased gradually from first fortnight of August to October and led to total (100 % yield loss) of the crop. In Abkhazia, brown marmorated stink bug Halyomorpha *halys* (Stål) (Heteroptera: Pentatomidae) was reported to reduce the yield of peach, mandarin, and orange by 13.2–87.4% when compared to the long-run mean of yield (Musolin et al., 2018). Fall armyworm (FAW) is another new invasive pest of corn. The annual damage that FAW does to sub-Saharan Africa's maize, rice, sorghum, and sugarcane crops is estimated to be at US$13 billion (Day et al., 2017). In East Nusa Tenggara of Indonesia, fall army worm is reported to cause damage to maize from 85% to 100% (Mukkun et al., 2021). While in Banten, the FAW larvae was found to damage paddy to a considerable extent , there wasn’t any record of damage by this pest to paddy in Indonesia (Sartiami et al., 2020).

Insect pest infestation is reported to damage coniferous trees too. The cones of *Pinus strobiformis* was damaged mostly by Lepidopterans (72%), while *Conophthorus ponderosae*, *L.*

occidentalis and *M. albifrons* accounted for 15%, 7% and 6% damage respectively (Leal- Sáenz et al., 2021). Hence, insect pests cause considerable damage to wide variety of crops, so immediate attention should be given to mitigate arthropod infestation.

Effect of soil on arthropods community

Soil along with providing essential nutrients to plants and promoting agricultural yield, also serves pivotal function of maintaining biodiversity (Pahalvi et al., 2021). Indeed, the soil host myriad of organisms of different shape, size, physiological activity, and ecosystem function (Doran and Zeiss, 2000; Ferris and Tuomisto. 2015). Much of the investigation and study has been carried out in earthworms; however, in terms of quantity, diversity, and range of niches they occupy, arthropods also have considerable significance in soil environment (Edwards, 2004).

Changes in soil's physical, chemical, or biological qualities can have an impact on biodiversity, abundance, and functional interactions among arthropods (Parisi et al., 2005; Sapkota et al., 201). For example, soil pH, soil texture and vegetation cover can influence the number of proturans, from few hundred to thousands (Galli et al., 2019). For instance, changes in soil pH can directly impact the availability of food sources and influence the overall soil microenvironment, which in turn affects proturan populations (DeMarco et al., 2011) and also soil texture influences its water-holding capacity, drainage, and aeration, which are crucial factors for proturan survival (Culliney, 2013). In one study, Christian et al., 2004 found the absence of proturans from all arable lands when agrochemicals were introduced in soil and when there was repeated mechanical disturbance. However, Diplurans can readily avoid mechanical disturbance and thrive easily with increase in soil depth where there is constant supply of moisture (Christian & Szeptycki, 2004). In Lepidopterans (such as Fall army worm), pupation take place deep inside the soil, and larvae were found to pupate more when 25–50% moisture was present in soil, compared to dry (5%) or wet (80%) soils (Shi et al., 2021). Similarly, in Hymenopterans (such as ants), soil moisture and soil disturbance were found to affect the distribution and abundance of ant community; there was low number of ants when dry soil was present and vegetation cover was reduced (Schmidt and Diehl, 2008). In Hemipterans (such as aphids), increased soluble nitrogen level in soil can result in its higher density and greater infestation to crops (Zehnder, 2015). It is because in nitrogen rich soil, there will be changes in plant physiology that makes plants attractive for aphids and aphids also reproduce vigorously in nitrogen rich plants (Sudderth et al., 2005). Dipterans are considerably affected by the physicochemical properties of soil. Their lifecycle at some stage depends on soil and can be divided into three categories: those which dwell in soil for entire life, dipterans that spend immature stage in soil, and those which pupate in soil (Frouz, 1999). Presence of soil organic matter, increase in soil moisture, addition of minerals and reduced soil disturbance by tillage facilitates abundance of dipterans larvae and increased oviposition of female adults (Weber, 1993). Also, deposition of heavy metals and soil acidification is found to alter the community of dipterans (such as Chironomidae, Sciaridae, psychodidae etc) (Frouz, 1999). It is reported that the decrease in humidity, increase in soil temperature, and increase in soil bulk density lowers Isopterans (such as termite) activity (Pribadi et al., 2011). Subphylum Myriapoda consists of Chilopoda, and Diplopoda class arthropods that are considerably affected by soil parameters as they complete most of their lifecycle in soil (Menta & Remelli, 2020). For instance, increase in soil pH is found to decrease Diplopoda (like millipede) community activity-density. The reasons are multifold: an increase in soil pH can disrupt the microbial community in soil which reduces the food availability for millipedes and also higher pH can increase soil toxicity and affect millipedes' survival and reproduction (Stašiov et al., 2021). However, other arthropod community activity-density was found to increase when pH of leaf litter was increased (Stašiov et al., 2021). Like Diplopoda, Chilopoda (like centipede) community composition is significantly altered by change in soil pH and soil moisture (Klarner et al., 2017).

Thus, by affecting nutrient availability, organic matter content, soil texture, pH, and soil chemistry, soil parameters and physicochemical properties of soil play direct and indirect role on the growth, development, and overall abundance of arthropods in given ecosystem.

Application of Biochar on arthropods' control

Globally agricultural production is constrained by insect pests. Insect pests are reported to cause the most serious and alarming yield losses in crops (Dhaliwal et al., 2010; Barros et al., 2014). One of the pressing challenges today is to find new ways to mitigate insect pest population. Sadly, biochar's impacts on managing insect pests have gotten far less study than those of diseases. Biochar effect on beneficial insect pest like bees and bumble bees hasn't been studied yet; nevertheless, biochar has attracted significant research and commercial interest on harmful insect pest management these days.

By enhancing the physical, chemical, and biological characteristics of the soil and subsequently creating a healthy environment for plants to grow and develop, biochar can be considered a useful tool for increasing the systemic resistance of plants against insects. The use of biochar in controlling plant herbivores may be due to its porous structure, which protects the growth of the soil microbiome, as well as its alkaline pH, which promotes the availability of nutrients (Noyce et al., 2015; De et al., 2021). Many research and observations have been done regarding effect of biochar on agriculture crop protection, but still we have limited knowledge on how biochar applications can impact herbivores and influence plant-herbivore interaction.

Biochar has been shown to boost plant resistance to insects and has been reported by Elad et al. (2010) to alter the ecology of insect pests and their interactions with plants.

In their study, Tonnang et al. (2022) hypothesized that an increase in atmospheric carbon dioxide (CO_2) concentration might impact insect ecology, physiology, metabolism, and consumption, which in turn increases their population densities and causes more crop damage and yield loss. This shows that a major decrease in atmospheric CO_2 may greatly reduce the prevalence of insect pests. Biochar may be the best candidate to reduce CO_2 content and enhance CO_2 sequestration because of its porous carbon-based adsorbents that has capacity of CO_2 physisorption through van der Waals force on the surface of the materials. Chemically, two amine functional groups on biochar can chemically react with one CO_2 atom through zwitterion mechanisms, producing carbamate and ammonia pairs (Choi et al., 2009; Rashidi et al.,2016; Jung et al., 2019). Because the carbonaceous materials in biochar have potential for CO_2 physisorption, their CO_2 adsorption capacity increases as pressure that is emitted during pyrolysis increases (Oschatz et al., 2018). Hence, it can be suggested that biochar can have an indirect role in controlling insect pests. In an experiment, Elad et al. (2010) reported that biochar applied on soil considerably reduced the occurrence of the expansive mite pest (*Polyphagotarsonemus latus* Banks) on pepper by providing systemic resistance to plants against insects and diseases. Biochar smoke water (SW), a bubbling smoke obtained during pyrolysis of five organic feedstocks (i.e., cellulose, wood sawdust, olive mill residues, maize, and alfalfa litter) at two temperature 300 and 500 °C in distilled water showed repellent effect towards invasive olive fly (Bactrocera olieae) on fresh olive plant after conducting bio-assay experiment (Bonanomi et al., 2021). Giunti et al., (2018) in their research found change in behavior of *Tribolium confusum*, stored-product coleopterans when it is exposed to Volatile Organic

Compounds (VOCs), and it was hypothesized that it took place due to its olfactory sense. Biochar smoke has been very effective to repel true flies, especially insects belonging to the order Diptera. Biochar smoke, however, can't be a feasible medium to control insect pests because of chemical constituents of it. One study conducted by Fabbri et al. (2013) postulated that biochar smoke water because of its acidic nature and presence of polycyclic aromatic hydrocarbon, exhibited phytotoxic effect on plants making plant weak and susceptible to various types of insect pests.

The effect of biochar on sucking insect pests has been explored a few times in the past. In one such experiment to examine the effect of biochar on fecundity of brown rice planthopper (*L. striatellus),* it was found that corn biochar applied at 30 g/kg concentration gave rise to higher fecundity than rice and wheat biochar. When the concentration was decreased to 50 g/kg biochar level, fecundity interestingly decreased more in corn biochar compared to rice biochar and wheat biochar, suggesting that increased biochar concentration reduces the fertility rate of brown rice planthopper (Qiang et al., 2018).

On one study of effect of biochar on brown plant hopper, it was found that *Nilaparvata lugens* nymphal development time was delayed, nymph-to-adult survival decreased, egg hatching rate was slower, and herbivore lifetime fecundity was decreased at a high amount of 200 g/kg biochar treatment compared to other amounts of biochar (0,10, and 50 g/kg) on rice seedlings (Hou et al., 2015). This may be due to biochar stimulating general defense pathways in plants associated with decline in Nitrogen and increase in Silicon amount. This finding contradicts that of the other study by Viger et al. (2015) that both Arabidopsis and the crop plant lettuce (Lactuca sativa L.) experienced vigorous plant growth after following biochar application but observed decreased regulation of a high amount of plant defense genes.

EPG (electrical penetration graph) method was used to investigate the probing and feeding routines of two major insect pests: the English grain aphid Sitobion avenae on wheat, and Laodelphax striatellus, a little brown planthopper, on rice, when grown in soils with varying biochar treatments. It was found that these insect pests had difficulty penetrating the stylet on their host plants and is proposed due to decrease in stimulatory Nitrogen and increase in inhibitory silicon (Chen et al., 2020).

Soil arthropods are very important in maintaining the soil ecosystem by nutrient transformation, breakdown of debris and decomposition of soil organic matter. They have an important role to play in making soil fertile but can also poses detrimental effect on plants and yield (Eisenbeis et al., 2012). One of the most researched soil arthropods on which biochar effect was measured, was spring tails. One study revealed that the reproductive rate of springtails (Collembola) was lower, and mortality rate was increased with biochar amendment (Conti et al., 2018). The finding of this research contradicts another research by Marks et al. (2014) where he found that increase in biochar amendment can cause an increase in the fecundity rate of Collembola. These findings are contradicted by another study done by (Gruss et al., 2019) where wood-chip biochar was applied in oilseed rape and maize crops, and it was found that all kinds of springtail life-form groups (hemiedaphic, epedaphic, and euedaphic) were favored by biochar instead of damaging it after application of biochar in soil.

The biochar research on insects has also been expanded in study of forest insect pests. One study found that the survival rates of four different types of forest insects: (Ips pini (Say), Formica obscuripes Forel, Enoclerus sphegeus and bark beetle predators were considerably reduced when insects had direct contact with the material in higher biochar concentration (Cook and Neto, 2018).

Table 1. Effect of biochar on arthropod community

Biochar type	Arthropod species	Effect of biochar	Reasoning	References
Rice straw biochar	Termites (*Coptotermes formosanus*)	>5% biochar was found to repel termites and rate of survival was decreased. >10% biochar control termites.	rise in soil PH and decrease in soil humidity	Chen et al., 2022
chicken manure biochar	sawtoothed grain beetle (*Oryzaephlius surinamensis*)& American wheat weevil (*Rhyzopertha dominica*)	Mortality percentage of 86.66% in sawtoothed grainbeetle & 73.33% in American wheat weevil	decrease in soil moisture and insecticidal activity of biochar	Hassan et al., 2022
Corn biochar Wheat biochar Rice biochar	English grain aphid (*Sitobion avenae* Fab)	reduced aphid lifetime fertility, reproduction, and growth. Effect was pronounced at 5% biochar concentration.	Increased silicon availability in soils that provide resistance against insect	Chen et al., 2019

Table1, cont

Wheat biochar	rice leaf-folder (*Cnaphalocrocis medinalis*)	decreased mature larval body weight and decreased consumption of rice leaves	decreased quality of rice plants due to the reduction in nitrogen content in leaves	Chen et al., 2019
Deciduous tree wood	white-backed plant hopper (*Sogatella furcifera* Horváth)	impaired growth and development	higher level of jasmonic acid by biochar priming effect	Waqas et al., 2018
Conifer saw mill	Douglas-fir Tussock moth (*Orgyia pseudotsugata*)	Increased larval mortality and less survival	lower availability of Nitrogen	Rice-Marshall et al., 2021

The preference of arthropods could be affected through biochar which was dose dependent. There was no obvious preference shown by arthropods on low concentration of biochar, but arthropods did not choose the soil that has been treated with high dose of biochar and hence forced to relocate (Cook et al., 2018). Arthropods would also escape from soil that had been treated with biochar because these compounds are highly hygroscopic and would cause the soil to dry out (Baronti et al., 2014).

Despite the potential advantages of application of biochar, its effects seem to be diversely present on the arthropods (Table 1). Through the application of biochar, studies found that the reproductive rate of arthropods decreased, and their mortality rate increased (Domene et al., 2015). However, Marks et al. (2014) found that biochar can stimulate the reproduction rate of arthropods through maintaining the biochar rate.

Thus, biochar has been more effective in controlling insect pests to a considerable extent, but it may differ according to the concentration of biochar used, types of insect pests, and stages of insects where biochar is applied.

Associated toxicity and risks of using biochar

Although much research has shown biochar to be effective against pathogens, weeds and insects as well as in promoting soil nutrient retention, soil conditioning, increasing water holding capacity, promoting enzymatic activity of microbes, and ensuring maximum productivity of crops, it comes with the cost. Biochar also has been getting wide attention due to the environmental hazards and risk that it poses to the ecosystem and organisms of all levels, considering that toxic organic pollutants present in biochar may enter the environment (Ruzickova et al., 2021). It has been demonstrated that high concentration of biochar in clay soils has detrimental effects such as decrease in available water content and in sandy soil, there is an increase in erosion by application of biochar on surface region (Khaledi et al., 2023).

Biochar contains toxic compounds that must be taken into consideration due to their negative impact on both target and non-target species. There have been reports of negative effects on reproduction, growth, and DNA integrity in earthworms, as well as a change in the fungi-to-bacterial ratio (Brtnicky et al., 2021). This thing should be cautiously considered before applying biochar on soil. Furthermore, biochar has a large range of physiochemical characteristics, including the size of biochar particles (Prodana et al., 2019), high pH, salinity (Lehmann et al., 2011) which affect normal growth and development of plants. In addition to it, toxic elements present in biochar can cause inhibition of nutrient absorption and photosynthesis process (Nagajyoti et al., 2010) along with providing toxic effects to the species (Ren et al., 2018). Only considering the advantages of using biochar is insufficient. The usage of biochar should be examined from all angles. The biochar use and demerits depend upon method of preparation of biochar, soil type and type of organic waste used in preparation. It is important to conduct several experimental studies on biochar considering both the short- and long-term impacts on the environment as well as on both plants and microbes (George, 2022). If these things can be addressed effectively then biochar can be effectively incorporated on a large scale.

Conclusion and future directions

The population growth rate is exploding globally, and food production has been adversely affected and dwindling due to a plethora of reasons, one of the primary reasons being insect pest infestation in crops. Chemical fertilizers and pesticides are being used to control harmful arthropods but in vain, as it has caused a multitude of problems such as soil acidification, nitrification, desertification, etc., to name a few. Hence, there is an urgency in the development of sustainable management strategies such as biochar that can benefit plants greatly by enhancing soil physiochemical properties as well as facilitating the control of insect pests by examining their role in insect traits. Recent studies and research in biochar effect on arthropods have provided ample opportunity to scientists to tease apart composition and mode of action of biochar specifically. While biochar has gained a lot of attention for its use in controlling weeds, and pathogens, they have also been an area of interest for studies in insect-plant interactions. However, this review explicitly explains how the properties of biochar have an immense effect on arthropods of different classes, having different feeding behavior and different behavioral ecology. Primarily, the high carbon content, porosity of biochar, and its alkaline PH are found to deter herbivory by mechanisms such as reduction in Nitrogen, increase in Jasmonic acid, increase in Silicon content, increase in soil moisture, decrease in soil moisture content, upregulate plant defense genes etc. Also, insects with sucking and piercing as well as biting and piercing mouth parts have been found to be adversely affected by the biochar application. Whether biochar has a direct (contact) effect on herbivores or facilitates an indirect (systemic) effect on insect pest eradication by improving soil properties or by strengthening plant defense genes is yet remains to understand it better to find the answers. More research should also be done on the effect of biochar in pollinator communities, predators, and parasitoids as well as entomopathogenic fungi as none of the research has been done in this area. More research should also be done on different crops and groups of plants to access the relative benefits of biochar among crops. Researchers should focus on examining which life stage of insects is more affected by biochar and specifically what group of insects face adverse effect by biochar. Also, more research is necessary due to the paucity of studies on creating methods for generating biochar inexpensively, testing it as a biopesticide, and replacing chemical insecticides with it for sustainable agricultural production. The cost-effectiveness of biochar along with its insecticidal properties can easily make it a suitable candidate for the Integrated Pest Management technique. Research in these areas will provide an avenue to plant ecologists or entomologists to further explore the application of biochar in plant-insect interaction and the behavioral ecology of insects. We urge our fellow scientists to move beyond traditional sustainable management practices and give biochar a try to better understand, quantify, and prepare biochar and examine in detail their role in mediating these interactions.

CHAPTER II

BIOCHAR REDUCES THE PUPATION AND ECLOSION OF A SPECIAIST (MANDUCA SEXTA) AND A GENERALIST (SPODOPTERA FRUGIPERDA) PEST

Abstract

Biochar is a charcoal-like substance made by the pyrolysis of organic material from agricultural and forestry waste. While biochar is well documented for altering soil physicochemical conditions, only a few studies have investigated its possible effects on the management of arthropod pests. Tobacco hornworms (*Manduca sexta*) and fall armyworms (*Spodoptera frugiperda*) are specialist and generalist insect herbivores respectively, that can cause significant defoliation in natural and agricultural ecosystems. Most studies on anti-herbivore defenses have focused on either larval or adult life stages, ignoring the less mobile and more vulnerable pupal stage. Since biochar contains compounds that can cause cytotoxicity in insects, it is plausible to expect that biochar can potentially affect their pupation and eclosion. To test this, we performed lab experiments with modified pupation media enriched with walnut shell biochar, allowing the insects to pupate and eclose. Biochar treatment on *M. sexta* pupae led to a reduction in pupal mass, and pupal deformity compared to the control, and these negative effects cascaded to significant reduction in the body mass of adults. We also found that adult wingspan, body length, and survival were also significantly lower in the biochar treatment when compared to the control. Similar results were also observed in the generalist pest *S. frugiperda*. We conclude that biochar negatively impacts the pupation and eclosion of lepidopteran herbivores and may have the potential to be employed as a management tool. However, further investigation with varying biochar concentrations and additional herbivore species is warranted.

Introduction

Biochar is a product of pyrolysis of organic waste such as crop residues, husks, and seeds under conditions of restricted oxygen (Chan et al. 2007; Pereira et al. 2016; Wang et al. 2019; Fan et al. 2021). The use of biochar as a valuable soil additive has gained worldwide attention in recent years because of its potential use in long-term carbon sequestration (Smith 2016; Sheng et al. 2016), soil fertility improvement (Manirakiza and Sekar 2020; Khan et al. 2021), soil nutrient acquisition (Abbas et al. 2019; Egamberdieva et al. 2020; Allohverdi et al. 2021), reducing nutrient leaching loss (Hardie et al. 2015; Alkharabsheh et al. 2021), weed control (Arif et al. 2012; Yavari et al. 2022), yield (Xu et al. 2021; Kapoor et al. 2022), and soil microbial community promotion (Ren et al. 2022; Bonanomi et al. 2021). Although the application of biochar in plant protection has only been popular in recent years, we are starting to see evidence for biochar in suppressing plant diseases (Jaiswal et al. 2019; Rasool et al. 2021), suggesting possible roles in other areas of plant protection, including anti-herbivore defense.

Chemical insecticides are used widely to control arthropods; however, the excessive use of chemical insecticides is not only expensive for the producers but can result in the degradation of soil through the inhibition of microbial and enzymatic activities and reduce the diversity of beneficial arthropods including decomposers, pollinators, and predators (Jallow et al. 2017; Mostafalou and Abdollahi 2017). Furthermore, in some cases repeated application of chemical insecticides has resulted in an increase in the prevalence of resistance in insects (Umina et al. 2018; Rezende-Teixeira et al. 2022). Clearly, alternate methods of pest management are in high demand (Singh et al. 2022). Due to the above, examining and developing biochar-based pest management strategies might be of interest to both researchers and producers. Interestingly, few studies have examined the potential role of biochar as an anti-herbivore defense, affecting herbivore survival and/ or reproduction.

Addition of biochar to soils has been reported to negatively affect the sap-sucking broad mite (*Polyphagotarsonemus latus* Banks) (Elad et al. 2010), reduce fertility of the brown rice planthopper (*Laodelphax striatellusas*) (Qiang et al. 2018), delay nymphal development and reduce adult fertility of brown plant hoppers (*Nilaparvata lugens*) (Hou et al. 2015), reduce fertility and growth of the English grain aphid (*Sitobion avenae* Fab) (Chen et al. 2019), impair the growth and development of the white-backed plant hopper (*Sogatella furcifera* Horváth) (Waqas et al. 2018) to name a few. In addition to their effects on sucking herbivores, a few studies have also reported that biochar can affect chewing herbivores; these include higher mortality in sawtoothed grain beetle (*Oryzaephlius surinamensis*) and American wheat weevil (*Rhyzopertha dominica*) (Hassan et al. 2022), decrease in survivorship and ability to repel termites (*Coptotermes formosanus*) (Chen et al. 2022). However, concerns regarding spraying or mixing biochar with other chemical and cultural management strategies may have hindered additional research, as biochar can have toxic effects on non-target species, and even inhibit plant growth and development (Nagajyoti et al. 2010; Ruzickova et al. 2021).

More than 85% of all holometabolous herbivores undergo pupating phase in soil (Whiting, 2002), and soil properties such as soil pH, depth, moisture, and temperature can control pupal growth and development (Khan and Usman 2020; Amaral et al. 2021). For instance, the higher soil moisture negatively affects the pupation and eclosion of *Spodoptera frugiperda* (He et al. 2021; Shi et al. 2021). Moreover, higher soil depth and too much soil moisture negatively affects the pupation and eclosion of Oriental fruit fly, *Bactrocera dorsalis* (Hou et al. 2006). Intervention at the pupation and eclosion stage can have significant effects on pest management, especially when pupal stage is considered the most vulnerable stage in life cycle (Portman et al. 2020; Singh and Kariyat 2020; Bawa et al. 2021; Singh et al. 2022). However, some studies (Lindstedt et al. 2019; Singh et al. 2022) have also found that pupae can also defend themselves against biotic and abiotic stressors.

Combining these premises, we tested whether biochar, as a soil amendment, can act as a pest control tool for the pupal stage of insect pests, thereby eliminating the non-target toxic effects of direct spraying. We also tested whether generalist and specialist herbivores would be differentially affected (if any) by the biochar treatment as they are the two significant holometabolous pests causing huge economic loss on crops. We used the Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), which is a polyphagous herbivore attacking at least 353 host plants (Singh et al. 2020; Hardke et al. 2015; Fajemisin et al. 2023) and tobacco hornworm *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae) is an oligophagous herbivore attacking Solanaceae family plants (Kariyat et al. 2019; Portman et al. 2020; Kariyat et al. 2018). Both lepidopteran herbivores pupate in the soil. Impacts on pupation time and pupal traits can affect adult fitness traits (Kariyat et al. 2019). We hypothesized that biochar would negatively affect pupation success considering toxic chemicals present in biochar along with its insecticidal property documented in prior research, and these effects would be more severe in the specialist herbivore, due to their inability to process toxins outside their host range, possibly leading to cascading effects beyond pupation and eclosion success.

Materials and methods

Insects and diet

The eggs of *M. sexta* were purchased from Great Lakes Hornworm Ltd. Romeo, Michigan, USA, and reared under laboratory conditions while eggs of *S. frugiperda* were obtained from Frontier Agricultural Sciences company. The eggs were hatched under a lamp maintained at 40.5°C to fasten the hatching process (N=138 for both *M. sexta* and *S. frugiperda* larvae). They were reared on synthetic lepidopteran diet in plastic containers (Dimensions: 23.19 cm × 15.24 cm × 16.84 cm; Aquaculture pet carrier: # 564356887, Walmart) according to Tayal et al. (2020 a, b) and Singh and Kariyat (2020). After caterpillars of *M. sexta* as well as *S. frugiperda* reached the wandering/ prepupal stage (the final instar larvae when they stop feeding and search for the place to pupate), they were transferred to a special cage set up differently for *M. sexta* and *S. frugiperda* as for differences in size (explained in detail in 2.4), and different measurements were taken accordingly.

Wood shavings as pupation media

To assess the direct effect of biochar on the pupation and eclosion of *M.sexta* and FAW, a control pupation medium i.e., wood shavings was used in the experiment. Wood shavings from Natural Aspen small animal bedding (Petco Animal Supplies, Inc., San Diego, CA, USA) were used as the medium for pupation. It was used solely for control treatment, and it was mixed with biochar at different concentrations to serve as different treatments, and wandering larvae were placed individually in each compartment and were observed regularly for the effect of biochar.

Biochar

Biochar was produced by gasification of walnut residue shells at a commercial farm and processing facility in Winters, CA, USA. Firstly, walnut shells were subjected to a downdraft gasifier supplemented with primary air and heated at temperatures between 150°C and 500°C, to form charcoal, gases, and tars. Gases and charcoal obtained were heated from 500°C to 900°C with a small addition of air to form syngas along with biochar (for further details, see Pereira et al. 2016).

Table 2: **Properties of walnut shell biochar used in the experiment (Pereira et al. 2016)**

Biochar properties	
Production temperature (°C)	900
pH	9.7
Ash content (%)	40.4
Total Carbon (%)	55.3
Hydrogen: Carbon	0.22
Particle size distribution	
>2mm(%)	43.6
1-2 mm (%)	19
0.25-1 mm (%)	15
<0.25mm (%)	22.4

Preliminary experiment

A preliminary experiment was designed to identify any possible pitfalls and logistical constraints if any, using *M. sexta* prepupal caterpillars. The preliminary experiment included control (with wood shavings only) and treatment (wood shavings mixed with 10% biochar). Wood shavings and biochar were incorporated in four layers: the bottom layer of wood shavings (100g), followed by wood shavings+ biochar (5%), then wood shavings (100g), and again wood shavings+ biochar (5%) on the top layer. For each treatment and control rearing container, two

M. sexta pupae were placed 2cm below the surface. The number of eclosed/uneclosed pupae and the number of deformed/normal adult moths were then examined. It was found that the percentage of eclosed pupae from control and treatment were 28.125% and 21.875% respectively. Also, the percentage of deformed pupae from control was 6.25% while it was 56.25% from biochar treatment. It clearly lended us with the idea of adverse effect of biochar and motivated us for conducting the main experiment. Pupae were considered deformed if they failed to successfully pupate, started to leak hemolymph, and died, or could not eclose. See figures 1a and 1b for the commonly observed deformities during the experiment.

Main experiment

To carry out the main experiment, eggs of *M. sexta* were allowed to hatch under laboratory conditions (at temperature 73°F and humidity 55%) in Edinburg, Texas, USA and the first instar caterpillars were moved into petri dishes and fed with artificial diet. A total of 138 larvae were used for experiments. For more details on *M. sexta* rearing, please see Chavana et al. 2021; Watts and Kariyat 2021. Once the larvae reached the wandering stage in the late fifth instar, they were removed from diet and were moved into plastic containers (dimension: 23.19 cm × 15.24 cm × 16.84 cm; Aquaculture pet carrier: # 564356887, Walmart). Each container was divided into two compartments with a cardboard sheet inserted in the middle of the box. After randomly assigning a compartment for control (only wood shaving (100g)) the other side was filled with wood shavings, with 10g of biochar equally distributed in 4 layers (as explained in 2.4.1.a.). Afterwards, 35 wandering caterpillars were placed in both treatment and control sides 2 cm below the surface and were allowed to pupate whereas other remaining fresh pupae was also placed in the similar way as wandering caterpillars. After 3-4 days, the pupae were carefully removed and, pupal sex (Singh et al. 2022) was determined, and pupal mass (initial mass; W1) was measured on a digital balance and then were put back in the treatment cages (Accuris Series Dx, Model: W3100-210, Benchmark Scientific, NJ, USA). After 12 days (16 days after placing them for pupation), the final mass (W2) was measured. To allow the pupae to successfully eclose (~18-21 after pupation; Tayal et al. 2020b), a cage was made using white corrugated cardboard folded and tied with Organza net and was placed over pupae in each compartment so that the eclosed adult moths could climb up the cage and expand their wings individually. Adult traits were observed twice a day (morning and evening), until emergences ceased. Following this, adult moths from treatment and control were isolated and their duration of deformity (duration of pupa placement till they are deformed), length of survival (duration from eclosion of moth to death), wingspan (measurement of width between the apices of the two wings), body length (length from tip of head to tip of abdomen of moths) was measured. Body length and wingspan was measured using a ruler (Ruler, White Vinyl: # 70260, North Carolina Biological Supply Co., NC, USA).

Experimental design in fall armyworm (FAW)

For FAW (N=138 larvae), a similar procedure (as explained in 2.4.1.b) was employed with modifications for their size (Singh and Kariyat 2021), and since FAW pupae are much smaller than those of *M. sexta*, we used a different sized cage. We also included an additional treatment with a biochar concentration of 5% to account for the small size of FAW pupae. For the cage setup, insect mesh cages (Outdoor Collapsible Insect Mesh Cage Terrarium Pop-up with Zipper Protection: Patio, Lawn & amp Garden, USA) was used. Each cage was divided into different compartments separated by cardboard sheets that is fitted tightly inside the cage. The treatments were designed in a way that,

Compartment 1: control (wood shavings only), Compartment 2, treatment 1: biochar concentration of 5% mixed with wood shavings, was used in 4 layers as explained in (2.4.1.b),

Compartment 3, treatment 2: biochar concentration of 10% mixed with wood shavings, was used in 4 layers. This procedure was repeated for all cages. All data collection methods were like that of *M. sexta*, adjusted for FAW size and pupation behavior.

Statistical Analysis

We used the combination of Analysis of Variance (ANOVA) and the Kruskal-Wallis test (non-parametric test) depending on whether the data met normality assumptions. As the mass of adult moths eclosed (gm) and body length (cm) data (of *M. sexta*) followed normal distribution, a one-way ANOVA test (treatment type as a factor) was used for analysis. The pupal mass loss percentage was calculated according to the following equation (Singh et al. 2021).

$$Pupal\ mass\ loss\ percentage = \frac{final\ mass - initial\ mass * 100}{initial\ mass}$$

For all pairwise comparisons of treatments (control, treatment 1, and treatment 2) in the FAW experiment, the analyses were followed by post hoc Tukey's HSD test. One-way ANOVA was performed with treatment (control and biochar) and sex along with their interactions as explanatory variables while the response variables were duration of deformity of pupa, duration of eclosion, mass of adult moth, adult body length, pupal mass loss percentage and wingspan.For analyzing the duration of deformity/death of pupa and duration of eclosion of moths, paired t-test was used (for *M.sexta*) while ANOVA test was carried out for fall armyworm. Additionally, to analyze the difference in length of survival for both *M. sexta* and *S. frugiperda*, Kaplan–Meier Survival analyses with Log-Rank tests and Tukey–Kramer HSD test were used for comparisons at multiple levels (P<0.05). For the quantitative comparison, % difference between control and treatment was calculated by formula,

$$\frac{Mean\ of\ control\ (e.g.\ mass\ of\ adult\ moth) * 100}{mean\ of\ treatment}$$

Similarly, the % difference between control and treatment was calculated for all the measured pupal and adult traits.

Data sets were analyzed using the statistical software, JMP (SAS institute, NC, USA), and plots were built using GraphPad PRISM software (La Jolla, CA, USA).

Results

Effect of biochar on length of time in deformity/death of pupae

In *M. sexta*, paired t-test was conducted, and it was found that there wasn't significant effect of treatment on the duration of deformity of pupa (P=0.3801). In FAW moths, one way ANOVA test was performed, and it was found that the time duration for pupae to be deformed or dead wasn't significantly affected by biochar treatment (P=0.0665) compared to the control. Also, in FAW, pupae placed in 10% biochar concentration were deformed 3.1 days earlier compared to control while pupae on 5% biochar concentration deformed 2.36 days earlier compared to control. In *M. sexta*, pupae placed in treatment deformed 1.09 days earlier as compared to control.

Effect of biochar on mass loss of pupae

Kruskal Wallis test was performed to analyze the effect of biochar on pupal mass loss. A significant effect of biochar on pupal mass loss percentage (PML%) was observed in *M. sexta*; biochar treatment ($\chi2$= 17.0484, P<0.0001) as well as FAW; biochar treatment ($\chi2$= 18.5581, P<0.0001) (Fig. 3a and 3c). Further analysis was carried out in FAW through Tukey-Kramer pairwise comparisons which demonstrated that both (10% and 5%) biochar treatments had a significant effect on pupal mass loss percentage, compared to the control (10% treatment: P=0.0023 and 5% treatment P=0.0178), while no significant effect was seen when 5% treatment is compared to 10% treatment (P=0.7868) (Fig. 3c). The interaction of sex and biochar treatment, however, did not have any significant effect on PWL% (sex x treatment; F=0.0528, DF=1, P=0.8186) in *M. sexta* as well as FAW (sex x treatment; F=0.0528, DF=2, P=0.2001) (Fig. 3b and 3d). Also, in FAW, 5% treatment significantly decreased pupal moss loss by 62.7% while 10% treatment significantly decreased mean pupal loss by 57.6% when compared to the control. In *M. sexta*, biochar treatment caused reduction of pupal mass by 59.7% compared to the control.

Effect of biochar on the mass of adult moth eclosed

One-Way ANOVA revealed a significant difference among treatments in the mass of adult moths in the case of *M. sexta*; biochar treatment (F=3.9742, DF=1, P=0.0490) (Fig. 4a), and Kruskal-Wallis test also showed a significant effect of biochar in mass of adult moths in FAW moths; biochar treatment ($\chi 2$=6.4750, DF=2, P=0.0393) (fig 4(c)). Tukey-Kramer pairwise comparisons in FAW indicated that both (10% and 5%) biochar treatments had a significant effect on adult FAW moth mass, (P=0.0182 and P=0.0239) respectively compared to the control. A similar effect of biochar concentrations (5% and 10%) was found on the mass of adult moths eclosed (P=0.935). The interaction did not have a significant effect on adult *M. sexta* mass (sex x treatment; F=0.3463, DF=1, P=0.5576) (Fig. 4b) or in FAW (sex x treatment; F=0.0528, DF=2, P=0.2001) (Fig. 4d). Also, in FAW, 5% treatment significantly decreased adult moth mass by 137.71% while 10% treatment significantly decreased mean adult moth mass by 105.9% when compared to the control. In *M. sexta*, biochar treatment caused reduction of moth mass by 192.73% as compared to the control.

Effect of biochar on adult body length

In *M. sexta*, the body length of the adult moth was found to differ significantly among treatments after (F=4.6604, DF=1, P=0.0333) (Fig. 5a). In FAW adult moths, the Kruskal-Wallis test revealed that treatment moths (5% biochar and 10% biochar) showed a significant reduction in body length size compared to control moths ($\chi 2$=12.4861, DF=2, P=0.0019) (Fig. 5c). The pairwise comparisons through the Tukey-Kramer HSD test revealed that both 5% treatment (P=0.0030) and 10% treatment (P=0.0063) treatment showed a significant effect on the body length of FAW adult moth compared to the control while no significant effect was found between 5% biochar treatment and 10% biochar treatment on the body length of FAW moths (P=0.9979) (fig 5(c)). On the other hand, for *M. sexta* sex and treatment interaction was not significant (sex x treatment; F=1.6848, DF=1, P=0.1974) (fig 5(b)), and a similar non-significant effect of treatments on sex was found for FAW moths (sex x treatment; F=0.0528, DF=2, P=0.4674) (fig 5(d)). Also, in FAW, 5% treatment significantly decreased adult moth body length by 108.33 % while 10% treatment significantly decreased mean adult moth body length by 108.522 % when compared to the control. In *M. sexta*, biochar treatment caused reduction of mean moth body length by 105.23 % compared to the control.

Effect of biochar on the wingspan of moths

Kruskal-Wallis ChiSquare test showed a significant reduction through biochar treatment on the wingspan of *M. sexta* ($\chi 2$=8.2012, DF=1, P=0.0042) (Figure 6(a)) and also in FAW ($\chi 2$=12.8450, DF=2, P=0.0016) (Figure 6(c)). Additionally, the Tukey-Kramer pairwise comparison of treatments in FAW showed that the 10 % biochar treatment had a significant reduction in wingspan compared to both control and 5 % biochar treatment, with (P=0.0008 and P=0.0499) respectively (Figure 6(c)). The interaction between sex and biochar treatment did not have a significant effect on the wingspan of *M. sexta* moths (sex x treatment; F=3.5304, DF=1, P=0.0633) (Figure 6 (b)) (as well as in FAW (sex x treatment; F=0.0528, DF=2, P=0.7165) (Figure 6(d)). Also, in FAW, 5% treatment significantly decreased adult moth wingspan by 114.11 % while 10% treatment significantly decreased mean adult wingspan by 154.87% when compared to the control. In *M. sexta*, biochar treatment caused reduction of mean moth wingspan by 126.61% compared to the control.

Effect of biochar on the duration of eclosion

Duration of eclosion is the time taken from placement of pupae on treatments to the time when they are fully eclosed. In *M. sexta*, paired t-test demonstrated differences in the duration of eclosion of pupae between control and treatment (P=0.0489). Interestingly, in FAW moths, the length of eclosion was examined using one-way ANOVA test and eclosion length was found to be similar between treatments and control with (P=0.7110). Furthermore, in FAW, pupae placed in 10% biochar treatment took 0.8932 more days to be eclosed into adult moths compared to control while pupae from 5% biochar treatment took an additional 0.7057 days compared to control. On the other hand, *M.sexta* treatment moth took 2.2914 more days to be eclosed compared to control moths.

Effect of biochar on the duration of survival of moth

Kaplan–Meier survival analysis curves on survival of *M. sexta*, demonstrated differences in the duration of survival between control and treatment (P=0.0020) showing higher mean survival of adult moths in control compared to biochar treatment (Supplementary Files Fig. 9a). The duration of survival of eclosed moths in FAW, on other hand, was also significantly affected by biochar treatment (P<0.0001). There were significantly fewer moths surviving from the 10 % biochar treatment compared to the 5% biochar treatment and control (Fig 9(b)). Also, in FAW, eclosed moths from control on an average survived 4.683 days more compared to moths from 5% treatment, and control moth survived 5.069 days more compared to moth from 10% treatment. On the other hand, *M. sexta* control moth survived 2.6638 days more compared to treatment.

Discussion

To examine whether biochar affects pupation and eclosion success of two lepidopteran herbivores, *M. sexta* and FAW, we analyzed the mass loss of pupa, adult moth mass, adult moth survival, body length, wingspan, and presence of pupal deformity between control and biochar treatment. Our results show that for both moth species, biochar significantly hindered normal growth and development of pupae which further leads to unhealthy and very retarded moths. The similar effect of biochar was studied by Osei et al. 2020 on the nematodes in which they tested the effect of biochar on insect population density in the Okra field and found that biochar has the potential to decrease nematode population densities. Another similar study by Cook and Neto, 2018 showed that the survival rates of four different types of forest insects *Ips pini* (Say), *Formica obscuripes* (Forel), *Enoclerus sphegeus* and *Temnochila chlorodia* were considerably reduced when insects had direct contact with the material in higher biochar concentration. These findings broadly align with our results indicating that the insects could be adversely affected using biochar. However, here we specifically targeted the pupal stage, as it can be directly affected by modifying pupation medium through biochar addition.

Only a few studies have analyzed the effects of biochar application for arthropod pest management. In most cases, the mode of action is thought to involve priming of plant insect-defense responses (Hou et al. 2015). Tonnang et al. (2022) postulated that a higher pyrolysis temperature will result in increased CO_2 adsorption capacity. Walnut shell biochar may be the best material to reduce CO_2 content and enhance CO_2 sequestration because of its porous carbon-based adsorbents that have the capacity of CO_2 reduction and CO_2 physisorption through van der Waals force on the surface of the materials (Jung et al. 2019). Such reduction in CO_2 concentration might decrease insect population dynamics, as CO_2 is needed for the accumulation of starch and Nitrogen, and for the promotion of secondary metabolites that provide resistance against insect pests that may happen during moth development and hence negatively impacting insect ecology, physiology, and metabolism (Xu et al. 2019; Johnson et al. 2020). Hence, a reduction in CO_2 concentration by biochar could have affected pupal mass gain and caused pupal deformity as well (Tonnang et al. 2022).

Most of the previous research in biochar-herbivore interaction has been done on the incorporation of biochar on soil and in interaction with plants (Hou et al. 2015; Viger et al. 2015) and the negative effect of biochar on herbivores has been hypothesized to be due to the decrease in available nitrogen, decline in soil moisture, reduction in silicon content and by facilitating the growth of microbiomes (Noyce et al. 2015; De et al. 2020; Chen et al. 2020). Although, these effects of biochar can't be ignored, the influence of biochar on the herbivore pupal stage which came in direct contact with it is never being acknowledged and studied. Therefore, we performed our experiment in the lab using only wood shavings under controlled conditions to reduce the effect of other environmental variables and to assess direct impact of biochar on herbivores. From our results, we can expect that the loss in mass of the pupa in experiments is possibly due to direct corrosion effect created by biochar compared to the control. It is in accordance with research where it was found that the cytotoxic effect of biochar on cells of humans and mice took place due to the presence of low molecular weight aromatic compounds and high level of toxic chemicals in it (Sahu et al. 2014; Yang et al. 2019; Gelardi et al. 2019).

Additional experiments with biochar added to different soil types would be interesting to look at their differential effects.

Marshall et al. 2021 found that the larvae can also ingest biochar through diet. The biochar might have been incorporated into the gut and made it more alkaline and caused deformity as well as hindered normal eclosion process in pupae. This can happen at two stages of pupation; at prepupal wandering stage just before the caterpillar shrinks and hardens to pupate, and before eclosion where the exoskeleton softens and possibly allows biochar to be imbibed by the pupa. It might also be interesting to test if biochar can downregulate the genes involved in pupation. For example, miR-100/miR-317, and miR-100/miR-285 genes which have been involved in pupation and eclosion (Zhang et al. 2020) and might have caused deformity in pupae as well as in adult moths. These deformities/malformation in pupae and adult might also be due to insecticidal properties of biochar caused by production of pyroligneous acid in the pyrolysis of walnut shell biochar (chemical constituents like phenols) and their antibacterial and antioxidant properties (Jahanban -Esfahlan & Amarowicz 2018).

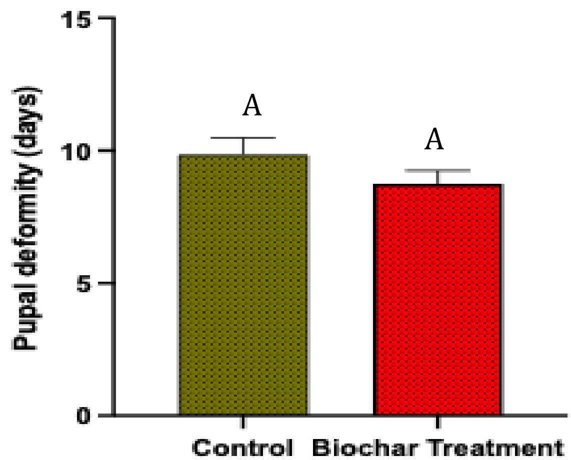
The adult moth and pupal deformity that we observed was comparable to deformities seen in other studies. For example., in-vitro incorporation of algal/cyanobacterial extract from *P. kessleri* and *N. carneum* exhibited the highest insecticidal toxic effects which negatively affected larval duration, % pupal formation, pupal duration, and mass, % moth emergence and pupal as well as moth deformity was observed in cotton leafworm *Spodoptera littoralis* developmental stage (Saber et al. 2018). They postulated that fatty acids such as the polyunsaturated ω-6 linoleic, palmitic, α-linolenic, and 7,10-hexadecadienoic acids displayed such insecticidal property that caused detrimental effect on cotton leafworm. However, Brtnicky et al. (2021) reported toxic effects of biochar on reproduction, growth, and DNA integrity in earthworms, as well as a change in the fungi-to-bacterial ratio. Also, biochar incorporation was shown to increase more larval growth and increase in fecundity of Collembola (Marks et al. 2014). This suggests that while biochar possesses the potential to be used as an effective Integrated Pest Management strategy, it could also pose several unforeseen yet severe impacts on other soil microbes.

While some researchers proposed the use of biochar in controlling the incidence of generalists and specialists like *M. sexta* and FAW, others suggest that use of biochar might not be feasible for large scale commercial farming. The findings from this experiment suggest that biochar could be used as a potential replacement for chemical pesticides and could lead to the practice of sustainable management of insects and pests. Although several studies present discrepancies in the efficacy of biochar as a pest management strategy, the current experiment reveals that biochar is indeed effective in controlling the eclosion of *M. sexta* and FAW which surmises that biochar at different concentrations can be an imperative strategy in realizing sustainable management of both generalists and specialist herbivores.

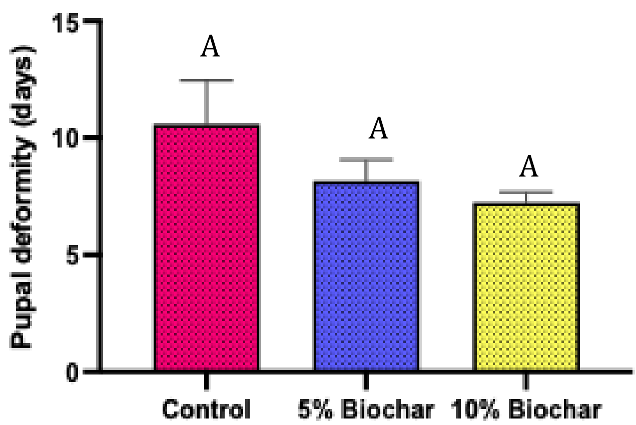
(a)

(b)

Figure 1. Common deformities observed on *M. sexta* pupae (a) and fall armyworm pupae (b).



(a)



(b)

Figure 2. The duration of deformity or death of pupae (duration of pupa placement in days till they are deformed or dead) in *M. sexta* (a) and FAW (b) in response to biochar treatments (no biochar control, 5% biochar, and 10% biochar) based on paired t-test (in *M. sexta*) *and* one-way ANOVA tests (in FAW). Means followed by different letters are significantly different ($P < 0.05$). Each error bar is constructed using 1 standard error from the mean.

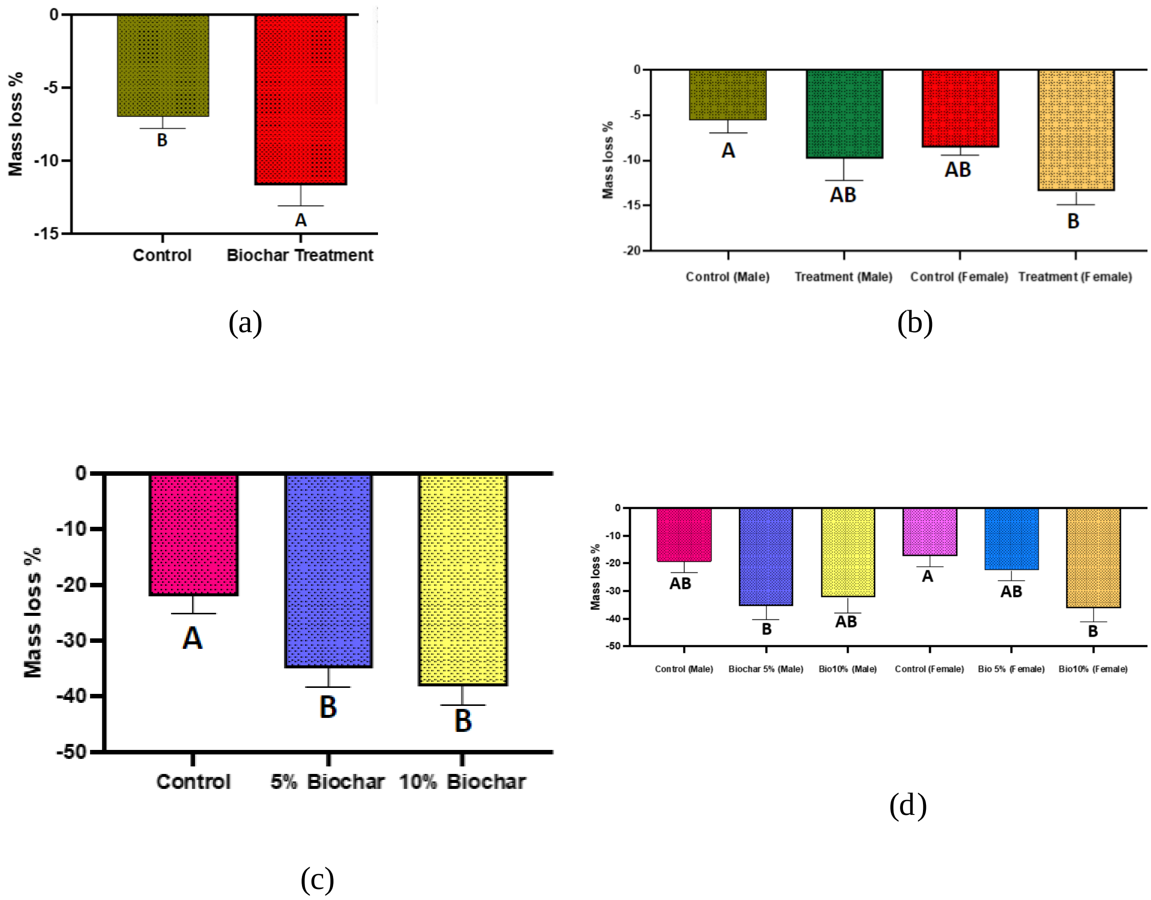
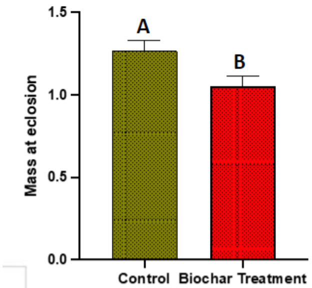


Figure 3. Pupal mass loss (%) in *M. sexta* and FAW (a, c) and by gender (b, d) in response to biochar treatments (control, 5% biochar, and 10% biochar) based on one-way Chi-Square tests. Means followed by different letters are significantly different (Tukey's HSD test, $P < 0.05$). Each error bar is constructed using 1 standard error from the mean.



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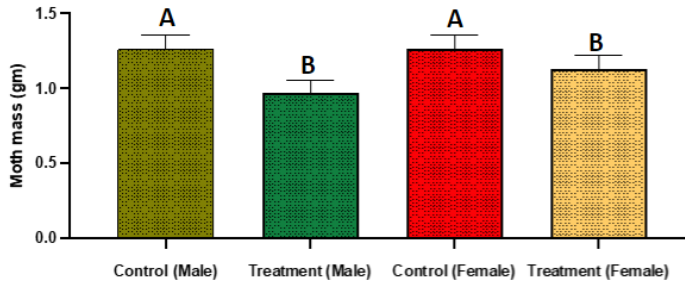

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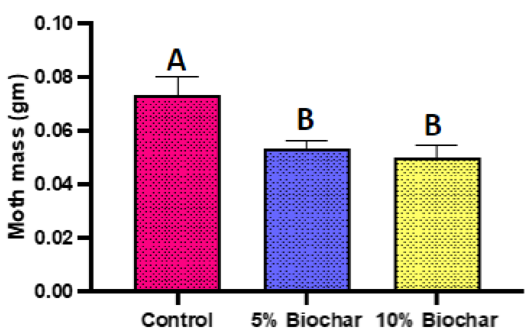
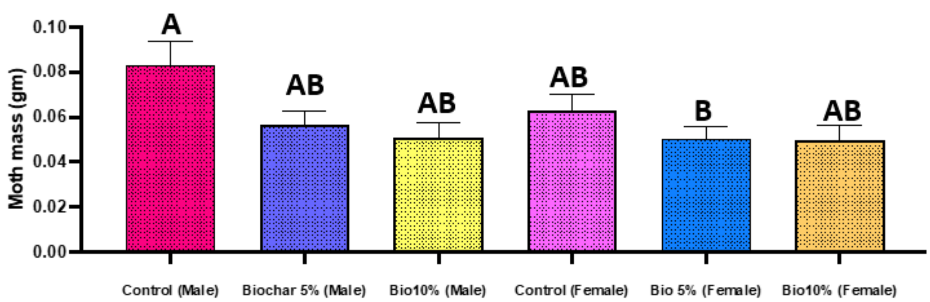
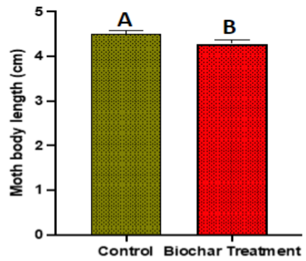


(c)



(d)

Figure 4. Mass of adult *M.sexta* and FAW (a, c) and by gender (b, d) in response to biochar treatments (no biochar control, 5% biochar, and 10% biochar) based on Kruskal-Wallis test and one-way ANOVA. Means followed by different letters are significantly different (Tukey's HSD test, $P < 0.05$)



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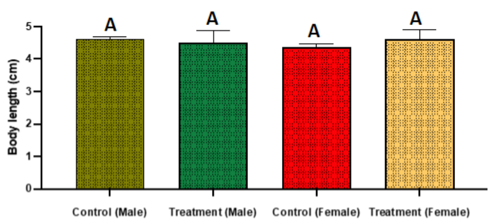

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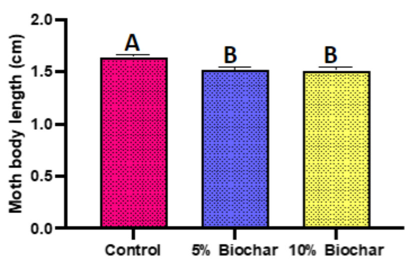

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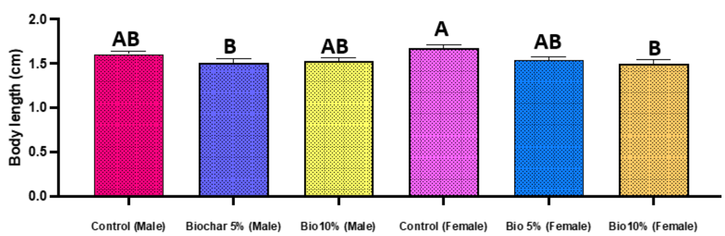
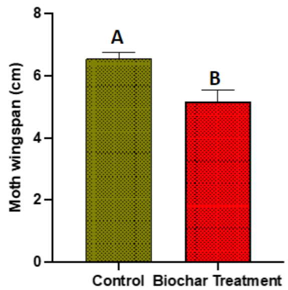


(d)

Figure 5. Moth body length of adult *M. sexta* and FAW (a, c) and by gender (b, d) in response to biochar treatments (no biochar control, 5% biochar, and 10% biochar) based on Kruskal-Wallis ChiSquare test and one way ANOVA. Means followed by different letters are significantly different (Tukey's HSD test, $P < 0.05$)



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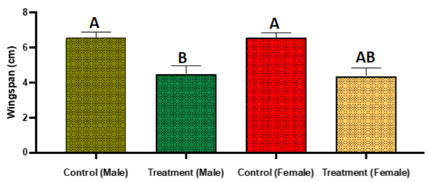

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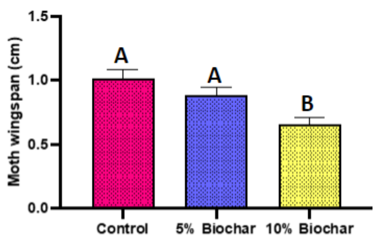

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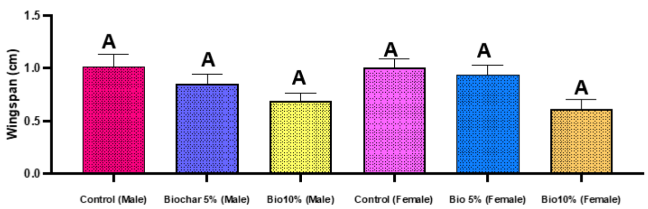
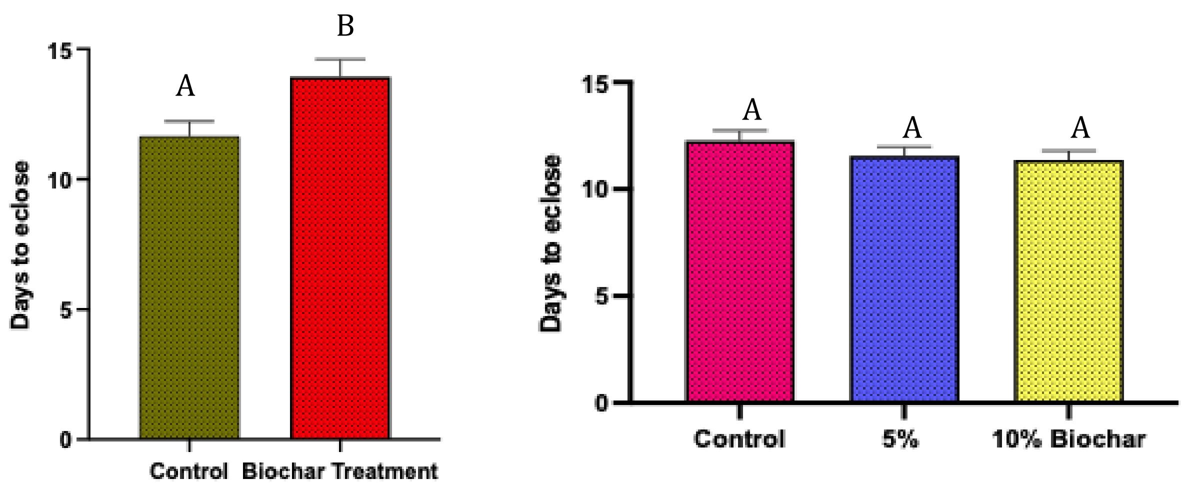


(d)

Figure 6. Moth wingspan in adult *M. sexta* and FAW (a, c) and by gender (b, d) in response to biochar treatments (no biochar control, 5% biochar, and 10% biochar) based on Kruskal-Wallis ChiSquare test. Means followed by different letters are significantly different (Tukey's HSD test, $P < 0.05$).

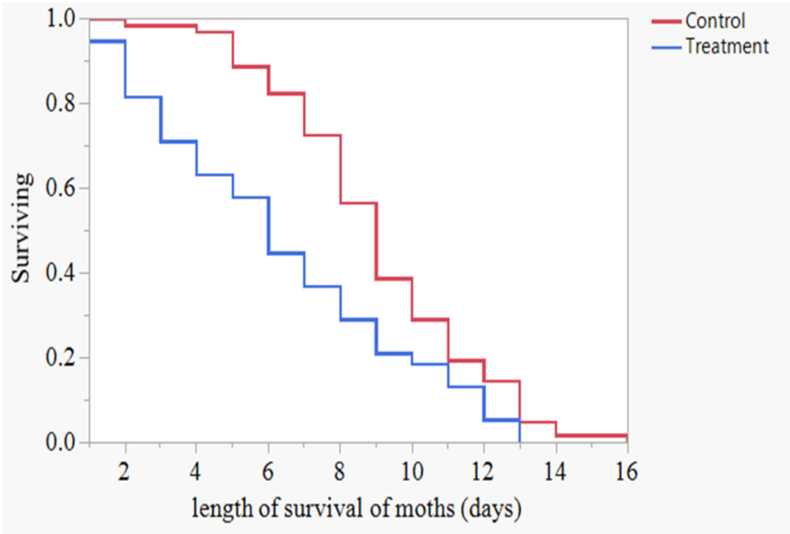
A **B** **C** **D**

Figure 7. Deformed adult moth with reduced wingspan and body length observed in our experiment in A (*M.sexta*) and B (adult FAW) compared to normal wood shavings eclosed moth, C (*M.sexta*) and D (adult FAW)

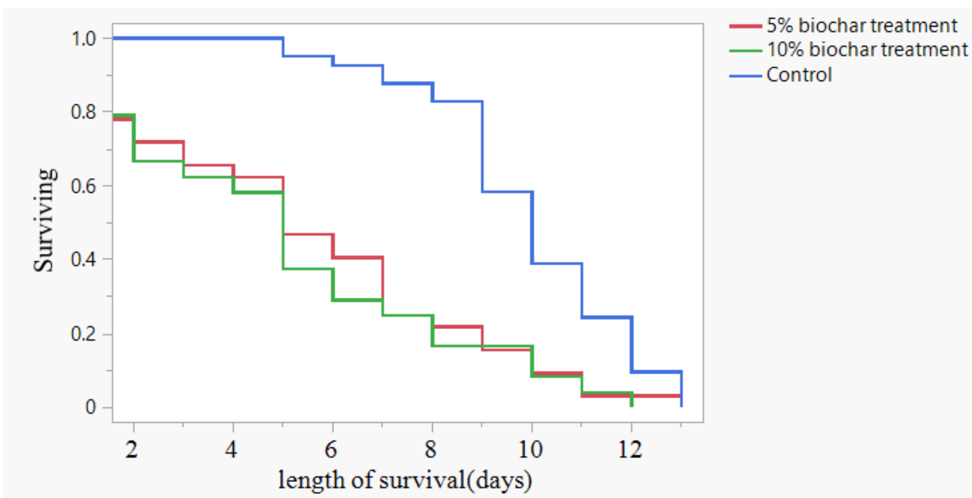


(a) (b)

Figure 8. Duration of eclosion of moth in *M. sexta* and FAW in response to biochar treatments (no biochar control, 5% biochar, and 10% biochar) based on paired t-test (in *M. sexta*) and one way ANOVA test (in FAW). Means followed by different letters are significantly different ($P< 0.05$)



(a)



(b)

Figure 9. Kaplan–Meier survival curves of (a) *M. sexta* adult moth duration of survival (days), (b) FAW adult moths' duration of survival (days). For each period interval, survival probability is calculated as the number of moths/pupae surviving divided by the total number of moths/pupae at the start of the experiment for each group of treatments.

CHAPTER III

CONCLUSIONS AND FUTURE DIRECTIONS

This thesis incorporates in-depth information about the concept of biochar, and how the biochar can detrimentally affect the pupation and eclosion success of a specialist (*Manduca sexta*) and generalist (*Spodoptera frugiperda*) pest. For this, the experiment was carried out to test the hypotheses that the amendment of a walnut shell biochar with wood shavings would adversely affect pupation and eclosion success and these effects will be more pronounced in the specialist herbivore possibly leading to cascading effects beyond pupation and eclosion success. As expected, biochar concentration of 10% was found to have detrimental effects on *M. sexta* and *S. frugiperda* resulting in 59.76% and 57.608% pupal mass loss, 192.73% and 105.96% reduction in mass of eclosed moth, their wingspan & body length was also significantly reduced resulting in greater number of deformed pupae, and prolonged time to eclosion. Although similar results were obtained with an additional 5% biochar concentration for fall army worm, the wingspan of the eclosed *S. frugiperda,* however, was not significantly affected by the biochar treatment. Based on current findings, biochar could potentially be used in the management of *M. sexta* and *S. frugiperda,* thereby elucidating the application of biochar as an effective IPM measure instead of chemical insecticides besides obvious benefits to crop productivity and nutrient enrichment in soil. These results suggest that biochar could be a useful alternative to chemical insecticides for managing pest populations and improving crop productivity. However, further research is needed to determine optimal application methods and to explore the use of biochar made from other materials. According to the results of this study, biochar was successful in hindering the pupation and eclosion of both *M. sexta* and *S. frugiperda* which suggests that widespread use of biochar, perhaps, could be an interesting add-on to current sustainable pest management strategies.

Moving forward, additional experiments should use various soil types instead of wood shavings, by modifying and controlling soil variables including soil microbiome, and other potential sources that can affect the physiochemical properties of soil including pH, nutrient content, organic matter content, moisture content, to name a few. Our controlled conditions experiment clearly shows the effects of biochar, and subsequent experiments can tease apart the details. Future studies should then be focused on distinct application methods of biochar in field conditions would be able to expand more on the successful use of biochar as a possible pest management strategy for generalists and specialists like *M. sexta* and *S. frugiperda*. At the same time, it is also possible to explore biochar made up of other materials (rice straw, wheat straw, corn biochar, oak wood) which might provide farmers with cheaper, easier, and accessible ways of utilizing biochar as an insect pest management technique. It has been well documented that most lepidopteran herbivores including *M. sexta* and *S. frugiperda* pupate during June-July months so biochar should be incorporated in soil during mid-May at optimum depth when excessive defoliation symptoms in plants are observed, particularly during summer season so that pupae can be deformed and reduce their eclosion and prevent future damage on plants.

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BIOGRAPHICAL SKETCH

Nischal Wagle completed his bachelor's study at Tribhuvan University, Nepal (2014-2018). He has been involved in many research projects and activities after his graduation. He had a strong interest and passion in entomology, so he joined Dr. Rupesh Kariyat's lab in the Spring of 2022 at the University of Texas Rio Grande Valley, USA. He has studied the effect of biochar on the pupation and eclosion of a specialist (*Manduca sexta*) and a generalist pest (*Spodoptera frugiperda*). He earned his master's degree from University Of Texas Rio Grande Valley in July 2023. Nischal Wagle will be joining the Krupke Lab at the Entomology Department at Purdue University for his Ph.D. in Entomology on fall 2023.

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