Response of Tomato to Different Concentrations of Papaya leaf extract as Bio-insecticide

BY

ALLEN PRINCY A.P

(20PBO001)

Thesis submitted to the

Avinashilingam Institute for Home Science and Higher Education

For Women, Coimbatore- 641 043.

In Partial Fulfillment of the Requirement for the

Degree of Master of Science in Botany

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A. Vijayalakshmi 20/5/22

Signature of the Head of Department

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Signature of the Supervisor

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CHAPTER 1

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae, genus *Solanum*, order Solanales and sub family Solanoideae (Idris *et al*., 2018). The species originated in Western South America and Central America.

Botanically described as fruits, but we use it as a vegetable. It contains vitamin C, potassium, folate, vitamin K, amino acid and antioxidant lycopene, which reduces cancers and neurodegenerative diseases (Idris *et al*., 2018). Regularly red in colour, but tomatoes have various colours like yellow, orange, green and purple.

Tomatoes are vital for its Umami flavour (Fleming and Amy, 2013). It can be taken in diverse ways, raw or cooked, in many dishes, sauces, salads and drinks.

Bio-insecticide

Bio-insecticides are the compounds that are used to control the insects, weeds in a natural or biological way which affects the plants. Currently, there are many types of bio-insecticides that are in use for the enhancement of crop. They are fashioned from animals, plants, microorganisms like fungi, bacteria, nematodes etc.

Need of bio-insecticide

Farmers rely on synthetic insecticides / chemical insecticide which are harmful to environment and soil. It also causes pollution. They are used for reducing the infection in plants and it gives healthier result, but it pollutes not only the environment, soil and plants, but also the consumers (human beings, animals) who feed on those plants and their products. It not only kills the targeted organism, but other beneficial non targeted organisms are eradicated.

The thrust to save the environment, soil and humans from the synthetic / chemical insecticides, the bio-insecticides had its origin. Bio- insecticides are eco-friendly, they help to avoid pollution. It helps maintain soil texture and the environment too. Bio-insecticides kills the specific / targeted organisms in a small group and it does not affect other organisms. The fruits or vegetables produced using bio-insecticide are safer, tastier and fresh.

The global market for bio-insecticide is treasured at 3.0 billion USD, accounting for 5% of the global pesticide market. With a yearly compounded growth rate of more than 15%, it is predictable that bio-insecticide market share will equal that of synthetic insecticides between 2040 and 2050 (Komivi Senyo Akutse *et al*., 2020).

Characteristics of bio-insecticide

Generally, bio-insecticide exhibit the following characters:

- tapered target range
- Highly definite mode of action
- restrain pests, not eliminate
- crucial timing of application
- inadequate field perseverance
- Short left over effect
- Safer to environment
- Safer to afford

Bio-insecticide from papaya leaf

Papaya plant has a rich source of vitamin C, A, B and E, magnesium and potassium minerals. It also contains cysteine, protease enzymes such as Papain and Kimopapain and compounds such as alkaloid, flavonoids and amino acid groups (Carrington, 2019).

Papaya leaf acts as toxicant and horrible against many insects which attack the plants. Papaya leaves are a natural insecticide to control the populations of German cockroach, *Coptotermes curvignathus Holmgren*, *Aedes aegypti*, *Rose aphids*, Pest in green mustard plant, a several viral diseases, *Paracoccus marginatus, Tribolium castaneum*, etc (Aditi Negi *et al*., 2021).

Commonly, one of the indicators to indicate the quality of agriculture is the pest free plants. Papaya leaf is used as medicine for dengue and some other *Aedes* affecting diseases. The bio-insecticide which is produced from the gum of papaya plant is very effective against insects. The gum which is obtained from a waste skin of a papaya plant is mixed with Natural Deep Eutectic Solvents (NaDES) which acts as a repellent. NaDES are bio-based ionic liquids that are composed of two or more compounds generally plant based primary metabolites such as organic acids, sugars, alcohols, amines and amino acids (Firda, 2017).

Some of the examples of bio-insecticides and their mode of actions are given in the following table.

Control agent	Mode of action	Examples	Control
Bacteria	It produces toxins which are harmful to certain insects	*Bacillus thuringiensis*	Lepidopterans
		Bacillus papilliae	Japanese beetle
		Agrobacterium radiobacter	Crown gall disease
Viruses	Insects which are feeding on plants will be stopped and when it is absorbed, it kills insects	Baculoviruses: Nuclear Polyhedrosis Virus (NPV)	Lepidopteran and Hymenopteran
		Baculoviruses: Granulosis Virus (GV)	Lepidopteran
		Baculoviruses: Group C *Entomopox*	Arthropods
Fungi	It weakens the insects outer coat and kills them	*Entomophaga praxibulli*	Grasshopper
		Zoophthora radicans	Aphids
		Neozygites floridana	Cassava green mite
Protozoa	When it is absorbed by plants, it kills the insects which are feeding on them.	*Nosema*	Grasshopper
		Vairimorpha	Lepidoptera
		Malamoeba	Locusts
Nematode	It enters into the cuticle of the targeted organisms body and kills them	*Heterorhabditis bacteriophora*	Black vine weevil, Japanese beetle
		Phasmarhabditis hermaphrodita	Various slugs and snails
		Steinernema carpocapsae	Black vine weevil, Strawberry root weevil, Cranberry.

Types of bio-insecticides

There are three major classes of Bio-insecticides. They are:

Microbial bio-insecticide

They are obtained from the microorganisms like fungi, bacteria, viruses and nematodes. These insecticides target specific or small groups of pests. These insecticides help to control large variety of pests. The most widely used microbial insecticide is produced from the bacteria *Bacillus thuringiensis* or Bt (Jitendra Kumar *et al*., 2021).

Substance found naturally

Insecticides are substance obtained naturally from the environment. These include plant products like garlic oil, pepper, etc. They do not kill the pest, but it controls the pest. The botanical extractions are protective against plant disease pathogens and other pests. (Jitendra Kumar *et al*., 2021).

Plants- Incorporated Protectants (PIPs)

Insecticides when incorporated to the plants using genetic engineering help to get modified in a natural way. This gives protection against insect pests. The insect's resistance genes are inserted into the DNA of the plants which get expressed in the plant and gives the required protection against insects (Muhammad Sarwar, 2015).

Importance of Tomato

- Tomatoes can assist persons with diabetes, prevent cancer, maintain healthy blood pressure and lower blood glucose levels.
- Carotenoids like lutein and lycopene are abundant in tomatoes. These can shield the eyes from damage caused by light.
- Tomatoes contain a lot of water and fibre, so they can aid with hydration and bowel movements.
- Vitamin C helps to keep skin, hair, nails and connective tissue healthy.
- It promotes bone strength since it contains vitamin K and calcium.

- The coumaric acid and chlorogenic acid found in tomatoes help to heal damages caused by smoking. Tomato juice helps to boost the immunity level of humans.
- Tomatoes encourage the production of the amino acid called carnitine, which helps to burn fat.

Importance of bio-insecticide

- Bio-insecticides are habitually intrinsically less toxic than conservative pesticides.
- Bio-insecticides generally affect only the target pest and closely related organisms, in contrast, conservative pesticides that may affect organisms as different birds, insects and mammals.
- Bio-insecticides often are effective in very small quantities and often decompose quickly, avoiding the pollution problems caused by conservative pesticides.
- When used as a component of Integrated Pest Management (IPM) programs, bio-insecticides can really reduce the use of conservative pesticides, while crop yields remain high.

Interaction between the plants and microorganisms is of four different types:

- Microorganisms can form symbiotic relationships with the host; these comprise bacteria like *Rhizobium* spp. and the rhizo-fungi.
- The microorganism may cause disease in the host plant;
- The host plant may act against the pathogen, no infection develops;
- The host plant may explain some acceptance to infection; in this case the pathogen is able to grow and imitate, but, symptoms of infection are least (Slater *et al*., 2009).

The pathogens are oppressed for natal control of insect pests through preliminary or inundative applications. In the recent years bio-pesticides are replacing the chemical pesticides to beat the harmful effect of the chemicals on non-target organism (Kachhawa, 2017).

The bio-insecticide industry in India is undergoing swift change, dazzling increased global trade in agricultural merchandise, a changing narrow environment and growing consumer preferences (Kiran Kumar *et al*., 2019).

The main objective of the present study is to show the importance of bio-insecticide from papaya leaf, which is easier to handle for the farmers and also cost effective.

CHAPTER – II

REVIEW OF LITERATURE

Jitendra Kumar *et al.* (2021) Their study indicates the utility of biocontrol agents composed of microorganisms including bacteria, cyanobacteria, and microalgae, plant-based compounds, and recently applied RNAi- based technology. This technology had been made for their application in modern agriculture for managing crop yield losses due to pest infestation.

Xiaoman Liu *et al*. (2021) proved that the biopesticide are safe to non-target organisms including humans. Compared with synthetic pesticides, new bio-pesticides can gain regulatory approval faster. They can also be developed in less time and are much less expensive. They concluded that the mode of action biopesticides should describe their current development for use in agriculture.

Sivakami and Renuka (2021) have shown that prepared iron oxide NPs by using *Murraya koenigii* leaves extract could be utilized as antibiotic drug and pesticides in biomedical and agricultural fields in future.

Davendra Pal Singh (2021) have reported that the bio-pesticides are eco-friendly pesticides which are obtained from naturally occurring substances, microbes and plants. However, in India, some of the biopesticides like *Bacilllus thuringiensis* (Bt), Nuclear Polyhedrosis Virus (NPV), *Beauvaria bassiana*, *Metarhizium* sp. and *Verticillium* sp. and neem-based pesticides etc. have already been registered and are being practiced. In this the Scientist attempted to highlight the role of biopesticides in agriculture and potential bio-pesticide available in India and how we can maximize the use of bio-pesticide for sustainable agriculture. He concluded that biopesticides play a vital role in controlling insect pests and maintaining quality of food for health of human beings and their livestock, environmental protection for sustainable development.

Ameilia Zuliyanti Siregar (2021) have shown that different types of arthropods present in wetland rice ecosystems play a role in biological balance to achieve environment friendly pest control towards sustainable agriculture. The potential of various types of natural enemies, especially the parasitoids and predators of brown planthopper pests and rice stem borer and preservation methods can be used as a case study of biological agents for the control of other rice plant pests.

Rani *et al*. (2021) in India have proved that the main challenge for bio-pesticide is related to their shelf life, narrow host range for pathogens, variations in the lab to land performances, economic regulations, etc. integrated approach will be beneficial for bio-pesticide application for this private and government sectors that come together with farmers to the village level and to build confidence in the use of bio-pesticides.

Sabi Mohamed Sani and Abdoul- Madjidou (2021) was conducted experiments as triplets treated with neem oil, and secondary treatment as NADIRA, HEINZI1370 and JAGUAR. They concluded that neem oil has given satisfactory results and may be recommended as an alternative against *Liriomyza* spp infested with Tomato.

According to Sharad Saurabh *et al*. (2021) Whiteflies are a group of universally occurring insects that are considered to be a serious pest in their own way causing both direct and indirect damages to crop. Countries should also increase their gross domestic product investment in R&D related to whitefly management based on GM/GE crops and/or nanotechnology to promote innovation, in the agriculture sector, where these technologies hold potential for 'Agriculture Revolution'. These multiple whitefly-centric strategies will ensure a successful campaign towards control of these tiny flies that are in actuality a mighty pest.

Bianca Guadalupe Flores Francisco *et al*. (2021) have concluded that agricultural production is one of the most important activities for food supply and demand that provides a source of raw materials, and generates commercial opportunities for other industries around the world. They also focussed on the importance of nematophagous fungi, particularly sedentary endoparasitic nematodes, on the development of biological control agents, the mass production of fungi *Purpureocillium lilacinum* and *Pochonia chlamydosporia*, and their limited commercialization due to the lack of rigorous method that enable the anticipation of complex interactions between plant and phytopathogenic agents.

De (2021) have said that Indigenous Technical Practices (ITP) are highly effective and viable for organic cultivation in North East India where use of inorganic fertilizers are used in limited quantities and organic inputs are available in plenty.

Rodrigo Fernandes de Oliveira *et al*. (2021) have shown that the use of EM (Efficient Microorganisms) applied to the soil controlled cabbage aphid and caterpillar and pepper extract controlled black spot. Forest litter, EM and bokashi were tested to control purple spot. Forest litter and bokashi together with EM controlled purple spot, but chive production was greater with the application of bokashi and EM. The research carried out could be considered an example of contextualized research, as desirable in agroecology. Applying EM to soil controlled the aphid and cabbage caterpillar and pepper extract had an inhibitory effect on black spot on papaya fruits. Applying forest litter and bokashi together with EM appeared to control purple spot on chives. However, chive production was greater with the application of bokashi and EM.

Maywan Hariono *et al*. (2021) have shown that *Carica papaya* (papaya) leaf extract has been used for a long time in a traditional medicine to treat fever in some infectious diseases such as dengue, malaria and chikungunya. The development of formulations for use in nutraceuticals and cosmeceuticals has caused this product to be more valuable nowadays.

Surya P. Singh *et al*. (2020) have reported that Papaya (*Carica papaya* Linn.) is well known for its therapeutic and nutritional properties all over the world. The different parts of the papaya plant have been used since ancient times for its therapeutic applications. The major findings of their study revealed that papaya leaf extract has strong medicinal properties such as antibacterial, antiviral, antitumor, hypoglycaemic and anti-inflammatory activity.

Resti Rahayu *et al.* (2020) has studied the potency of papaya leaf as insecticide to control the German cockroaches. The study aimed to determine the potency of ethanolic extract of papaya leaf as toxicant and repellent against populations of German cockroaches. They used contact toxicity test and repellency test. They concluded that the ethanolic extract of papaya leaf could be formulated as natural insecticide to control populations of German cockroach that have been resistant to synthetic insecticides. The study on the ethanolic extract of papaya leaf showed better toxicity and repellence against German cockroach. The ethanolic extract of papaya leaf residue was able to kill 50% if the range is 2.97 until 4.72mg cm-2 and if that is increased to 6.05 until 8.92 mg cm-2 could kill upto 90%.

Vimal Singh Rajput *et al*. (2020) have shown that there has been substantial renewal of commercial interest in biopesticides as demonstrated by the considerable number of agreements between pesticide companies and bio product companies which allow the development of effective biopesticides in the market.

Lukmanul Hakim Samada and Usman Sumo Friend Tambunan (2020) have shown that bio-pesticides play an important role in IPM by reducing the use of synthetic chemical pesticides that are harmful to human and environmental health. Some bio-pesticides dominate the global market, including Bt, neem, Baculoviruses and *Trichoderma* (fungicide). Regarding the resistance of pests, it is important to use suitable methods for pest control to increase agricultural yields. Biopesticides are becoming more widely used because of improved application methods, eco-friendly and cheaper options for many formulations. Therefore, bio-pesticides are a more rational choice for pest management, especially as an improved balance between cost and efficiency becomes a reality in the near future.

Nonice Manikome (2020) have proved that the combination of papaya leaf extract and soursop leaf extract were effective in controlling pests. Natural materials are easy to obtain and can be used as an environment friendly alternative for pest control.

Ameilia Zuliyanti Siregar and Yurnaliza (2020) have shown that rice and fish are the important source of protein in North Sumatra. They conducted research in rice comparing IR 64 Paddy types with Nila tilapia (*Tilopia mosambicca*). Different types of anthropods present in rice field play an important role in biological balance. The predators of brown planthopper pests and rice stem borer and preservation methods can be used as a biological agents for the control of other rice plant pests.

Yakobus Bustami *et al.* (2019) have done statistical analysis on the papaya leaf extract that possess a significant role in control of pests which attack the green mustard plant. It was effective against small nails, green grasshoppers and armyworms. The research method involved of 5 treatment and 5 replications with different concentrations of papaya leaf extract such as 0%, 25%, 50% 75%, 100%. In the 100% concentration of papaya leaf extract, the number of pests decreased to 0.40 compared to the concentration of 0%, which showed higher number of pests.

Kiran Kumar *et al.* (2019) have shown that microbial pesticides can play an important role in improving environmental and human health through the reduction of toxic chemical pesticides, which are used at alarmingly high levels in some regions of India. They concluded that microbial pesticide could increase the share of microbial bio-pesticides in the country.

Bipin and Dayanand (2019) have demonstrated the development of nano- pesticides, their properties, quality control, and synthesis techniques with reference to electro-spinning, delivery mechanisms, and effects and mode of action on insects for plant protection.

Oguh *et al*. (2019) have proved that Soil pollution and Air pollution occurring from the use of synthetic pesticides takes years and sometimes decades to break down the chemicals. People need to break the habit of using harmful pesticides and switch to bio-pesticides which break down quickly in sunlight and in the soil. The faster a chemical breaks down, the sooner the soil can return to a healthy state. He reviewed that, natural pesticides can easily be washed from fruits and vegetables making them healthier for us and our family to eat.

Budi Untari *et al*. (2019) have done research on physical interaction of chitosan-alginate entrapping extract of papaya leaf (*Carica papaya* L.) into submicron particles formation. Preparation of papaya leaf extract into submicron particle dosage form of chitosan and sodium alginate polymer using ionic gelation method aimed to increase the solubility of extract. The results of XRD revealed the changes of type of crystalinity form to amorphous on submicron particles. The results of FTIR revealed the physical interaction without chemical shifting.

Nismah Nukmal *et al*. (2019) have carried out studies on polar extract of gamal leaves (*Gliricidia sepium*) from various cultivars in Lampung, Indonesia and show a insecticidal effect on various types of mealy bugs, but its effect on papaya Mealybugs (*Planacoccus marginatus*) is not yet known. This study aimed to determine the insecticidal effects of water extract formulation of gamal leaf powder from four different cultivars, namely Bandar Lampung (BL) cultivars, Pringsewu (PR), North Lampung (LU) and West Lampung (LB) against papaya mealy bugs.

Chiga Sangma *et al*. (2019) conducted experiment on *Leucinodes orbonalis* Guen. and showed significant performance over untreated control. They concluded that the neem oil can be the effective insecticidal agent which when used will give a highest cost benefit ratio taking into consideration the serious health hazards caused by the chemical insecticides to consumers.

The raw material of bio-insecticide production can be derived from the abundant plant wastes and has a cysteine protease compound that can damage the tissue and digestive system in plant pests, namely papaya waste. Tibrizi Ahmad *et al.* (2018) The content of cysteine protease can be extracted using Ultrasound-Assisted Extraction (UAE) by varying sonication time and solid-to-liquid ratio with NADES solvent. The results indicated that NADES solvent proved to be efficient solvents. The process was carried out for 30 minutes which showed better result in killing the insects.

Microbial biopesticides include several microorganisms like bacteria, fungi, baculoviruses, and nematodes associated bacteria acting against invertebrate pests in agro-ecosystems. Luca Ruiu (2018) The author aimed to give light to new and increasingly effective microbial derived active substances.

Zikril *et al. (*2018) have indicated that the quality of agriculture should be proved along with the increasing quantity of agriculture in Indonesia. Generally one of the indicators or parameters commonly used to indicate the quality of agriculture is the number of pests that damage plants in certain areas. The control of disturbing organisms on the plant can be minimized by the bio-insecticide production from *Carica papaya* which is tapped and extracted with ultrasonic-assistance using a mixed NADES solvent from Cholin chloride. Bio-insecticide was produced by conducting qualitative enzyme activity using Lowry method and quantitatively using UV / Vis spectrophotometer aid with 750 nm wavelength and efficacy test. Based on the result of this research, the best extraction was found on NADES solvent with 1: 2 ratios with 30 minutes extraction time. Bio-insecticide samples effectively kill Grayak caterpillars with 100% mortality within 5-7 days.

Mohammad Kanedi (2018) have carried out research on the leaf extract of *Gliricidia sepium* and found that it could be used in contolling papaya mealybugs, *Paracoccus marginatus.* Bioassay was done by feeding mealybugs with young fruits of papaya that was previously soaked in aqueous and methanolic extracts of gamal for 10 minutes. Five levels of extract concentration were applied namely 0% (control), 0.05%, 0.10%, 0.15%, and 0.2%. Mortality of the insects were examined and assessed at the12th, 24th, 48th and 72nd hour. Aqueous and methanolic leaf extracts of gamal *Gliricidia sepium* exhibit lethal effect against papaya mealybugs *Paracoccus marginatus*. However, when both were compared based on the LC50, methanolic extract seemed to be little more toxic than that of aqueous. Overall, leaves extract of gamal is potent and could be used as a bio-insecticide in controlling papaya mealybugs.

Margaretta Christita dan Ady Suryawan *et al. (*2018) have compared the effectiveness of bio-insecticides made from papaya leaf extract, and chilli pepper in several different doses to reduce pest attack on white oyster mushroom (*Pleurotus ostreatus*). Experiments conducted on oyster mushrooms showed a significantly good result in terms of yield as well as effectiveness of insecticide when papaya leaf extract and chilli pepper were used.

Anondho Wijanarko *et al.* (2017) *Spodoptera litura* is one of the major pests on red chilli peppers *(Capsicum annum)*. Papaya latex could be used as a pesticide because it contains cysteine protease, which is a substance that can inhibit the insects from eating the leaves or even kill the pests. The enzyme activity test used a UV- is spectrophotometer and the efficacy test was done on *Spodoptera litura* larvae, which were given red chilli pepper leaf covered with an organic pesticide from cysteine protease. The latex extracted from the papaya bark shows higher activity in blending method than tapping method. The tapping method caused a decrease in enzyme activity compared to blending method.

Widya Hary Cahyati *et al*. (2017) have shown that *Aedes aegypti* mosquito population could be controlled by papaya leaf juice. The study was conducted to find out the chemical compounds in hay infusion and papaya leaf juice. They used phytochemical test using UV-Vis's spectrophotometry, Thin layer chromatography and High Performance Liquid chromatography (HPLC) method. They concluded that hay infusion and papaya leaf juice contains chemical compounds that could be used as attractant and bio-insecticide to *Aedes aegypti*.

Kachhawa (2017) have proved that biological control of pest and control of variety of diseases caused by different groups of microorganisms including virus, bacteria, fungi, protozoa and nematodes are important. In present day, development of resistance to chemicals and residue in higher tropical level are major hurdle in insect pest management. He concluded that the microorganisms provide certain distinct advantages over many other control agents and methods.

Anselm P Moshi and Ivy Matoju (2017) have reviewed and stated including relevant laws and regulations in order to establish the factors which have hampered legislative registration and commercialization of bio-pesticides in Tanzania. It is evident that some achievements have been attained by the indigenous people in using different types and forms of botanical materials in pest management, that have also been confirmed by research. They concluded that the plants which has the capacity to control insects used by indigenous people include Neem (*Azadirachta indica*), *Eucalyptus globules* leaf powder, Neem kernels, *Tephorosia vogelii*, *Euphorbia tirucalli* leaves and seeds, *Neurautanenia mitis* and *Pedilanthus cucullatus*.

Abdel-Tawab H Mossa (2016) studied the prospects of essential oils (EOs) as bio-insecticides for insect pest management. The EOs are a complex of chemical compounds with multiple modes of action that enhances their activity due to the synergetic action between constituents. EOs based insecticides are low toxic, environmental persistence and eco-friendly. He concluded that they are compatible with biological control programs and indigenous natural enemies of pests.

Sengottayan Senthil-Nathan (2015) aim on biopesticide research is to make these biopesticide products available at low cost, and this would become the possible tool in the integrated pest management strategy. He reviewed the important and basic defection of major pesticides in the past.

Biopesticides are the biological pesticides which can manage pest intervention through predatory, parasitic, or chemical relationships. Muhammad Sarwar (2015) Biopesticides have no harmful detection comparing with chemical pesticides. Biopesticides can be applied through introductions, augmentation releases, inundatively, or through conserving existing field populations of natural pest control agents. He concluded that Biocontrol agents can be used successfully against a complete range of high threshold pests including aphids, whiteflies, stem borers, leaf miners, locusts and grasshoppers.

Narendra Kumawat *et al*. (2014) have shown that now-a-days due to increase in chemical pesticides, farmers are switching towards organic farming, they are using biopesticides. Bio-insecticides can be prepared by using plants and waste materials. Conventional pesticides are generally synthetic materials that directly kill or inactivate the pest. They concluded that bio-pesticides play an important role in future pest control management.

Nayem Zobayer and Rokib Hasan (2013) have selected different concentrations of papaya leaf, neem leaf and garlic bulb extract to test pest control management in *Abelmoschus esculentus* (L.) Moench. In that they concluded that the neem leaf extract is outstanding than other two extracts.

Bacillus thuringiensis (Bt) bacteria are insect pathogens that rely on insecticidal pore forming proteins known as Cry and Cyt toxins to kill their insect larval hosts. Alejandra Bravo *et al.* (2013) The mode of action of the three domain Cry toxin family involves sequential interaction of these toxins with several insect midgut proteins facilitating the formation of a pre-pore oligomer structure and subsequent membrane insertion that leads to the killing of midgut insect cells by osmotic shock.

Srinivasan (2012) had reviewed on integrating biopesticides in pest management strategies for tropical vegetable production. Integrating biopesticide could enhance performance of IPM strategies. An IPM strategy based on sex pheromone for managing the eggplant fruit and shoot borer has reduced pesticide abuse and enhanced the activities of natural enemies including *Trathala flavoorbitalis* in Indo- Gangetic plains of South Asia.

According to Vigneshwara Varmudy (2011) papaya is a wholesome fruit having more carotene compared with other fruits. Carotene helps to prevent damage by free radical, which might otherwise lead to cancer.

Mamun and Ahmed (2011) have shown that biopesticides are secondary metabolites containing alkaloids, terpenoids, phenolics and renewable alternatives. Neem, Ghora-neem, Mahogoni, Karanja, *Adathoda*, Sweet flag, Tobacco, Chrysanthemum, Artemisia, Marigold, Clerodendrum, wild sunflower is used as tea pest controllers. These plants will control the undesirable side effects of synthetic pesticides.

Nwinyi *et al*. (2010) have studied the effects of extracts from *Carica papaya* L. on mycelia reduction of the most occurring fungal pathogen causing pawpaw fruit rot. Different fungi isolated were *Rhizopus* spp, *Aspergillus* spp and *Mucor* spp the aqueous seed extract and papain exhibited remarkable mycelia inhibition with mean zones of inhibitions between (0.23 – 1.73mm). They concluded that the *Mucor* spp exhibited a low level of mycelia reduction against the extracts when compared with the measurement of the extent of inhibition by the other extracts.

CHAPTER –III

MATERIALS AND METHODS

The plant taken for the current study was *Solanum lycopersicum* L. that belongs to the family Solanaceae. Bio-insecticidal spray prepared at different concentrations and the rate of control was measured along with the growth parameters in tomato plant.

Collection of seeds

Seeds of *Solanum lycopersicum* L. were obtained from Shri Sakthi store, Pappampatti, Coimbatore.

Morphology of the plant

Solanum lycopersicum L. **(Plate 1,2 and 3)**

Systematic Position

Kingdom - Plantae

Class - Magnoliopsida

Order - Solanales

Family - Solanaceae

Genus - *Solanum*

Species - *lycopersicum* L.

General description

- Tomato is a perennial herbaceous plant, but it is often grown as an annual crop.
- Tomato is cultured in tropical and temperate climates in open field. Green houses are habitually used for large-scale production.
- In warm climate with the right light intensity for growth, around 45 days are needed for germination and 90-100 days to attain the beginning of fruit readiness (OCED, 2017).
- The growth may attain up to 3 metres (m) in height.

- The primary root may grow numerous metres in length. The stem is angular and covered by hairy and glandular trichomes that bestow a characteristic smell.
- Leaves are tidy pinnately. Leaflets are petiolated and dentated. All leaves are roofed by glandular, hairy trichomes.
- The tomato fruit is globular or ovoid. The outer skin is a thin and fleshy tissue that comprises the remnants of the fruit wall as well as the placenta.
- The colour of the fruit is copied from the cells within the fleshy tissue.
- Tomato fruits can be either bilocular or multilocular.
- Between 50 and 200 seeds are located inside the locular cavities and are sheltered in gelatinous membranes.
- The seeds are small (5 x 4 x 2 mm) and lentil shaped.
- The seed contains the embryo and the endosperm and is enclosed by a strong seed coat called the testa.
- The progress of the fruit takes seven to nine weeks after fertilisation.

Plate 1 Habit of *Solanum lycopersicum* L.

Plate 2 Flower of Tomato

Plate 3 Fruits of Tomato

Taxonomy

- The Solanaceae, generally known as the nightshade family, also includes other well-known civilized plants such as tobacco, chilli pepper, potato and eggplant.
- Tomato categorization has been the topic of much debate and the variety of the genus has led to reconsideration of earlier taxonomic treatments.
- Tomato was originally named *Solanum lycopersicum* by Linnaeus in 1753; *Lycopersicon lycopersicum* L. by (Valdes and Gray, 1998).
- For a long time tomatoes were identified as *L. esculentum*, but latest research has revealed that they are part of the genus *Solanum* and are now over largely

referred to as *Solanum lycopersicum* (Spooner, Anderson and Jansen, 1993; Bohs and Olmstead, 1997; Olmstead and Palmer, 1997; Knapp, 2002; Spooner *et al*., 2005, 2003; Peralta *et al*., 2008).

Collection of materials

Coir pith, vermicompost and manure are collected from Kraft seed, Cloudtail India Pvt Ltd, India, Shipped by Amazon.

Grow bags from Forest Campus, IFGTB, Coimbatore.

Red soil from Kalangal, Sulur, Coimbatore.

Experiment in Grow Bags

The seeds were sown in 15 Grow bags (Plate 4 and 5) (30cm *30cm *45cm sized bags) containing red soil, coir pith, vermicompost and manure in the ratio 1:1:1:1. The treated bags were maintained in triplets. The tomato plant is sprayed with different concentrations of bio-insecticides prepared from papaya leaf to study about the rate of insect control, yield and growth of tomato plant.

The experiment was conducted during the 2022 dry season. Five different treatments of papaya leaf extract were used which includes Control (T_0), 25% papaya leaf extract (T_1), 50% papaya leaf extract (T_2), 75% papaya leaf extract (T_3), 100% papaya leaf extract (T_5). The soil, coir pith, vermicompost and manure mixture were filled in the grow bags. Each bag consists of 15 kg of mixture. The seeds of tomato were sown on 15th January 2022 and the seeds germinated on 25th January 2022.

Data collected includes number of leaves, number of fruits, number of flowers, number of insect before spraying and number of insect after spraying from 45th day. Papaya leaves and neem leaves were used to control insects. The insect started attacking the plant on 8th March 2022 ie., after 45 days.

The insect that attacked the tomato plant was **Mealybug**, belonging to the family Pseudococcidae, found in moist, warm habitats. They are considered as pests as they feed on plant juices and also act as vector for several plant diseases.

Bio-insecticide produced from papaya leaf

Bio-insecticide produced from *Carica papaya* L. (Papaya) shows more effective against many insects, diseases etc. the extractions produced from papaya leaves are mixed with solvents to produce insecticides (Ayun and Laily, 2015).

Systematic position

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Brassicales

Family - Caricaceae

Genus - *Carica*

Species - *papaya* L.

Preparation of papaya leaf insecticide

Materials required

Papaya leaf, Neem leaf, Water

PROCEDURE FOR MAKING PAPAYA LEAF EXTRACT

1. Cut 1kg of papaya leaves into small pieces

2. Papaya leaves that have been cut are then pounded to facilitate the extract.

3. The crushed papaya leaves are put into the drum, 10 litres of clean water is added and neem leaves were added.

4. After all the ingredients are added they are stirred well.

5. After 24 hrs of soaking, strain the water using a coconut milk filter to get papaya leaf extract.

6. Dilute papaya leaf extract with clean water according to the specified concentration (0%, 25%, 50%, 75% and 100%).

Treatment with different concentrations of papaya leaf extract

- 1st spray 25ml of papaya leaf extract was diluted in 1 litre of water and sprayed over the T_1 plants.
- 2nd spray 50ml of papaya leaf extract was diluted in 1 litre of water and sprayed over the T_2 plants.
- 3rd spray 75ml of papaya leaf extract was diluted in 1 litre of water and sprayed over the T_3 plants.
- 4th spray 100ml of papaya leaf extract was diluted in 1 litre of water and sprayed over the T_4 plants.

Harvesting was done 4 times at 7 days interval. The fruits started forming from 75th day. The first harvest was done on 1st April 2022.

Growth Parameters

The following growth parameters were calculated.

1. Number of leaves
2. Number of insects before spraying papaya leaf extract
3. Number of insects after spraying papaya leaf extract

Number of leaves

The number of leaves present was recorded in the uprooted plants.

Number of insects before spraying papaya leaf extract

The number of insects was counted in all the 5 treatments including control before spraying the bio-insecticide.

Number of insects after spraying papaya leaf extract

The number of insects was counted in all the 5 treatments including control after spraying the bio-insecticide.

Yield parameters

Number of buds

The number of buds formed in all the grow bags was calculated.

Number of fruits

The number of fruits obtained on 45th day, 60th day and 75th day were calculated for *Solanum lycopersicum* L.

STATISTICAL ANALYSIS

The data obtained from various morphological observations as well as yield parameters were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978).

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted in Tomato plant (*Solanum lycopersicum* L.) with a control and four different concentrations (25%, 50%, 75%, & 100%) (Plate 4) of Papaya leaf extract spraying to control the insect pests.

The germination and initial leaflet formation of the Tomato was observed (Plate 5). The parameters such as number of leaves , flowers, fruits, number of insects before spraying insecticides and number of insects after spraying insecticides were noted on the 45th (Plate 7) and 60th (Plate 8) days. Buds started forming on 35th day, so the number of buds formed was also measured on the 45th day. On the 45th day, the number of fruits formed was also measured and statistically analyzed. The yield parameters were statistically analyzed and the results are tabulated.

The insects started invading the tomato plant from the 45th day of growth of the plant. The papaya leaf extract at different concentrations were sprayed and it was found that in T_4 ie., 100% concentration, the insects were fully controlled (Fig 15).

Plate 4

Seeds of *Solanum lycopersicum* L. sown in grow bags

Plate 5

Seedling growth of *Solanum lycopersicum* L. after 10 days in control and treatments

Plate 6

Tomato plant on the 30^{th} day

Table 1

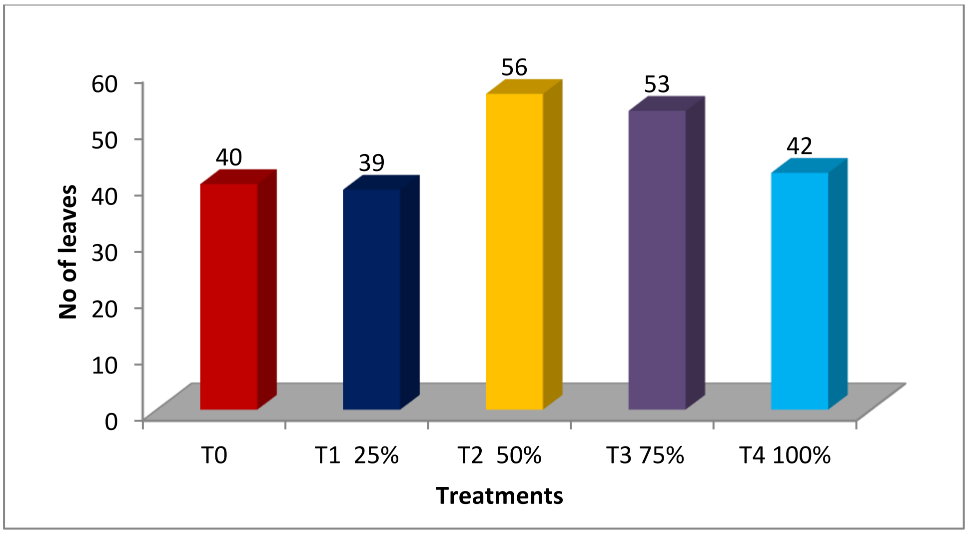
Insect control and yield parameters of *Solanum lycopersicum* L. on the 45th day.

Bags	Number of leaves	Number of flowers	Number of fruits	Number of insects Before spraying insecticide	Number of insects after spraying insecticide
T0	40 ± 4.5	**5 ± 0.9**	0	0	0
T1 25%	39 ± 4.2	0	**5 ± 0.5**	2 ± 0.6	2 ± 0.4
T2 50%	**56 ± 6.8**	4 ± 0.5	0	4 ± 0.3	3 ± 0.2
T3 75%	53 ± 6.9	2 ± 0.2	0	2 ± 0.1	1 ± 0.5
T4 100%	42 ± 5.4	3 ± 0.3	1 ± 0.5	**6 ± 0.8**	**1 ± 0.3**
SEd CD(P<0.04)	4.3453 10.0204	0.4453 1.0270	0.2614 0.6028	0.4282 0.9874	0.3000 0.6918

Plate 7

Growth of *Solanum lycopersicum* L. on the 45th day

Figure 1



Number of Leaves

T0 – Control

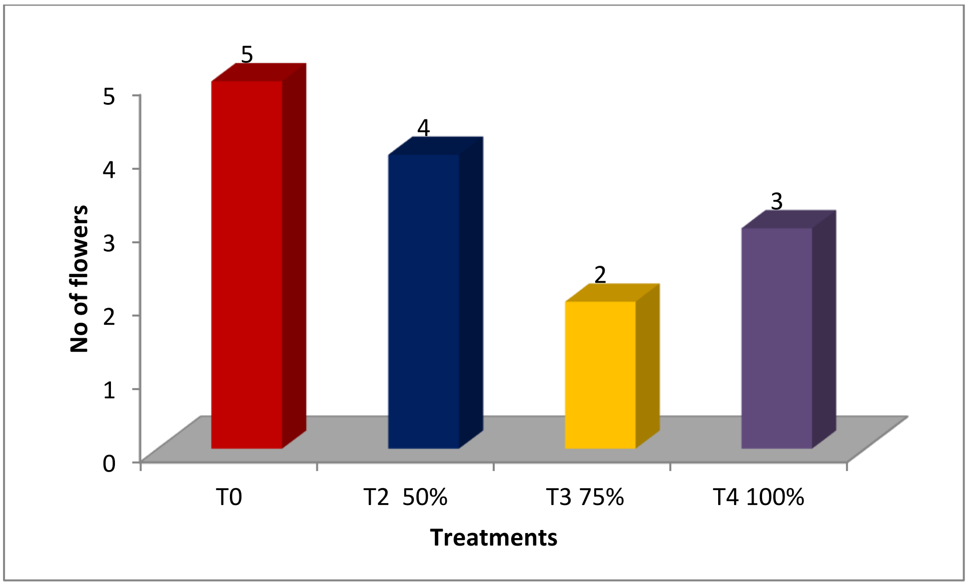
T1 – 25% of Papaya leaf extract

T2 – 50% of Papaya leaf extract

T3 – 75% of Papaya leaf extract

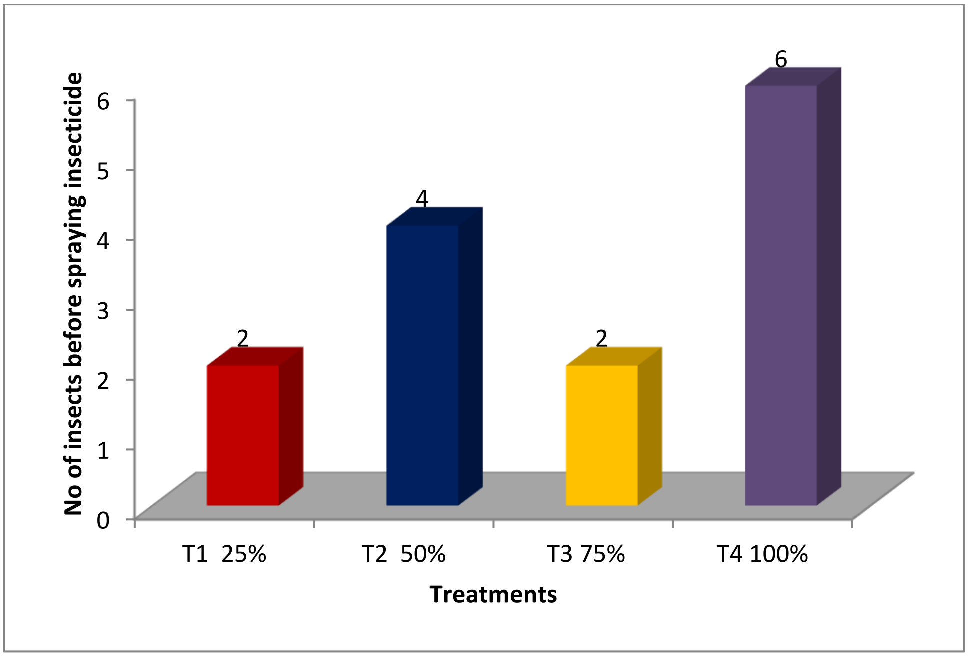
T4 – 100% of Papaya leaf extract

Figure 2



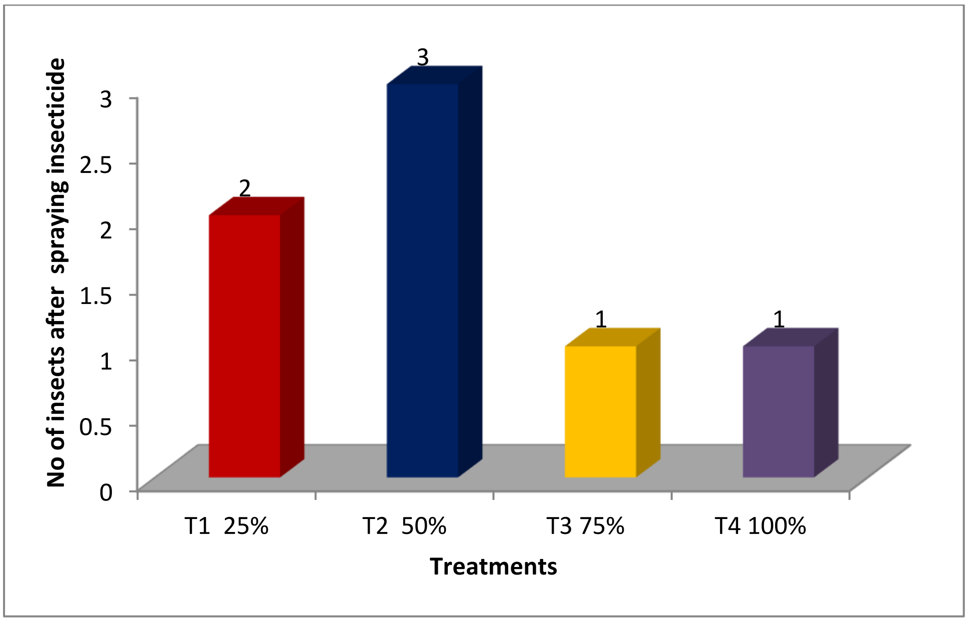
Number of Flowers

Figure 3



Number of insects before spraying insecticide

Figure 4



Number of insects after spraying insecticides

The Number of insects before spraying insecticides were found to be more (Fig 3) in T_4, but it got significantly controlled by spraying 100% papaya leaf extract. The number of leaves and flowers on the 45th day were higher in T_2 and T_0 respectively. The measurement observed were 56 ±6.8 (Fig 1) and 5± 0.9 (Fig 2).

Table 2

Insect control and yield parameters of *Solanum lycopersicum* L. on 60th day

Bags	Number of leaves	Number of flowers	Number of fruits	Number of insects Before spraying insecticide	Number of insects after spraying insecticide
T0	53 ± 6.8	5 ± 0.1	1 ± 0.3	20 ± 2.9	20 ± 2.3
T1 25%	43 ± 5.5	7 ± 0.6	6 ± 0.9	30 ± 3.4	26 ± 3.3
T2 50%	35 ± 4.4	9 ± 0.8	5 ± 0.5	25 ± 3.2	23 ± 3.6
T3 75%	65 ± 7.9	12 ± 0.3	7 ± 0.7	30 ± 3.3	21 ± 2.9
T4 100%	**76 ± 8.1**	**16 ± 1.4**	**9 ± 0.6**	**32 ± 3.5**	**9 ± 0.6**
SEd CD(P<0.04)	5.4635 12.1734	0.6387 1.4232	0.5164 1.1506	2.6671 5.9427	2.2483 5.0095

Plate 8

Growth of *Solanum lycopersicum* L. on the 60th day

Plate 9

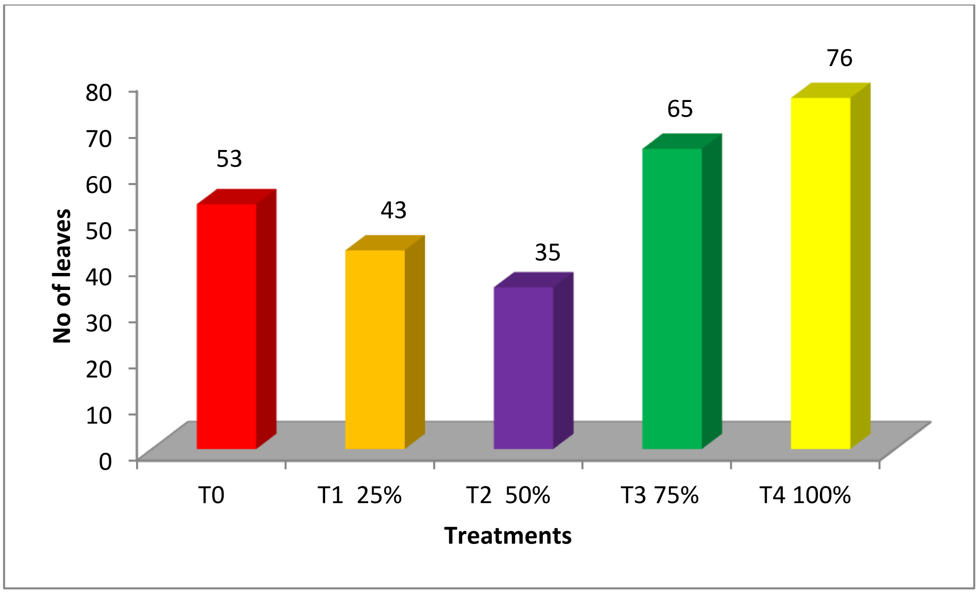
***Solanum lycopersicum* L. fruit**

The fruits started forming on the 60th day. The Papaya leaf extract does not affect the yield. The fruit was healthy and fleshy.

Plate 10

Number of insects on 60th day

Figure 5



Number of leaves

T0 – Control

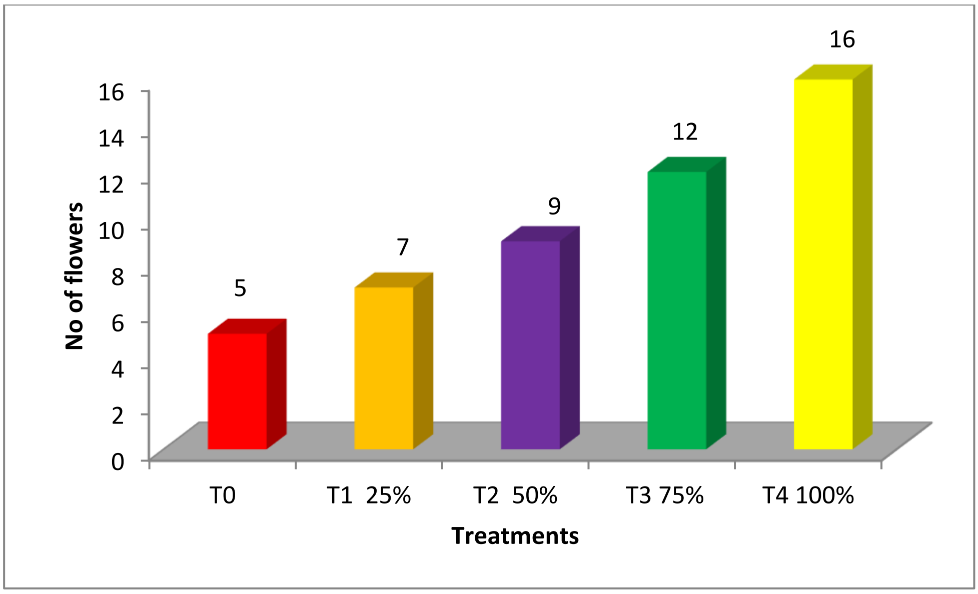
T1 – 25% of Papaya leaf extract

T2 – 50% of Papaya leaf extract

T3 – 75% of Papaya leaf extract

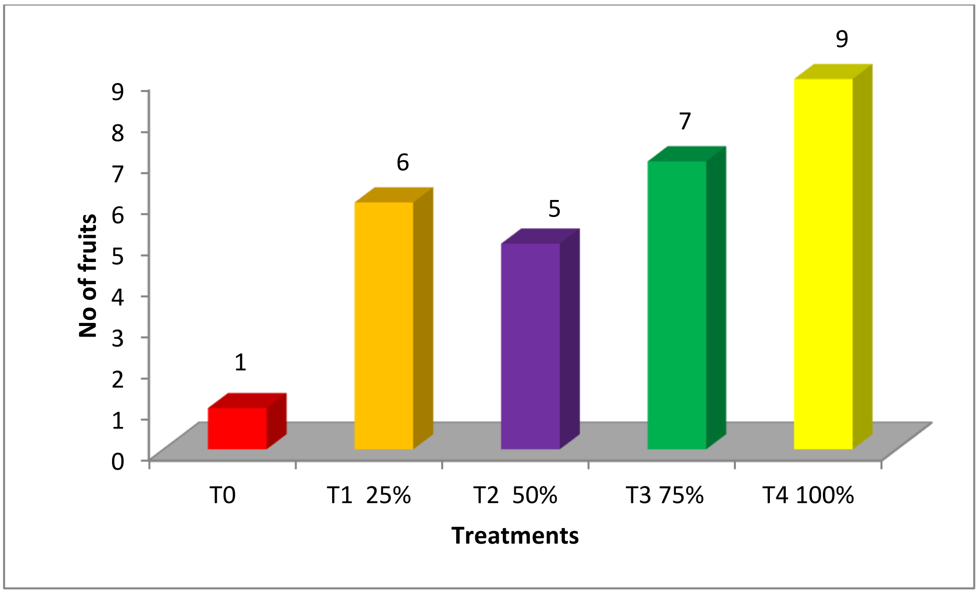
T4 – 100% of Papaya leaf extract

Figure 6



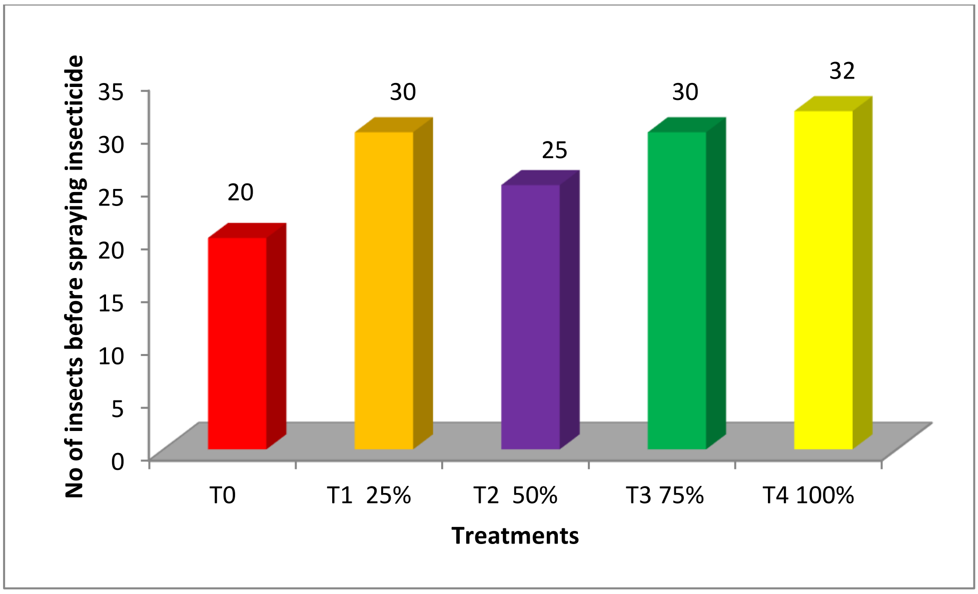
Number of Flowers

Figure 7



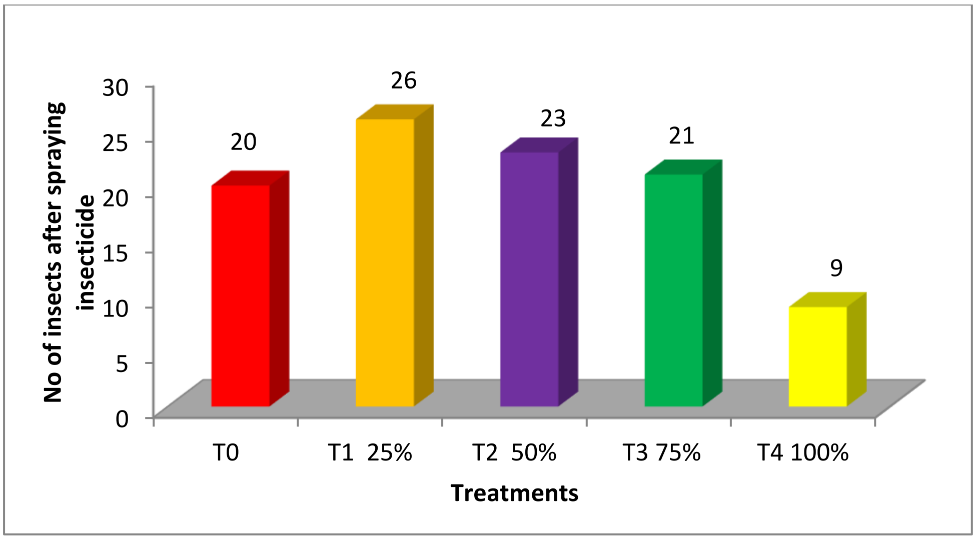
Number of Fruits

Figure 8



Number of insects before spraying insecticide

Figure 9



Number of insects after spraying insecticide

The Number of insects before spraying insecticides were found to be 32 ± 3.5 (Figure 8) on the 60th day. After spraying insecticide it decreased in T_4 (100%) concentration to 9± 0.6 (Figure 9). The number of leaves, flowers and fruits on the 60th day were higher in T_4, T_4 and T_4 respectively due to suppression of insect pests. The measurement observed were 76 ±8.1 (Fig 5), 16± 1.4 (Fig 6) and 9±0.6 (Fig 7).

Table 3

Insect control and yield parameters of *Solanum lycopersicum* L. on the 75th day

Bags	Number of leaves	Number of flowers	Number of fruits	Number of insects Before spraying insecticide	Number of insects after spraying insecticide
T0	54 ± 6.6	6 ± 0.9	5 ± 0.7	**39 ± 4.8**	39 ± 4.2
T1 25%	43 ± 5.8	8 ± 0.5	7 ± 0.4	26 ± 3.6	21 ± 2.1
T2 50%	35 ± 4.4	9 ± 0.3	8 ± 0.8	23 ± 3.3	18 ± 1.4
T3 75%	**65 ± 7.9**	**11 ± 0.6**	7 ± 0.1	21 ± 2.5	14 ± 1.8
T4 100%	54 ± 6.3	10 ± 0.2	**9 ± 0.3**	9 ± 0.3	**1 ± 0.6**
SEd CD(P<0.04)	5.1466 11.4675	0.4546 1.0129	0.4305 0.9592	2.6641 5.9360	1.9187 4.2751

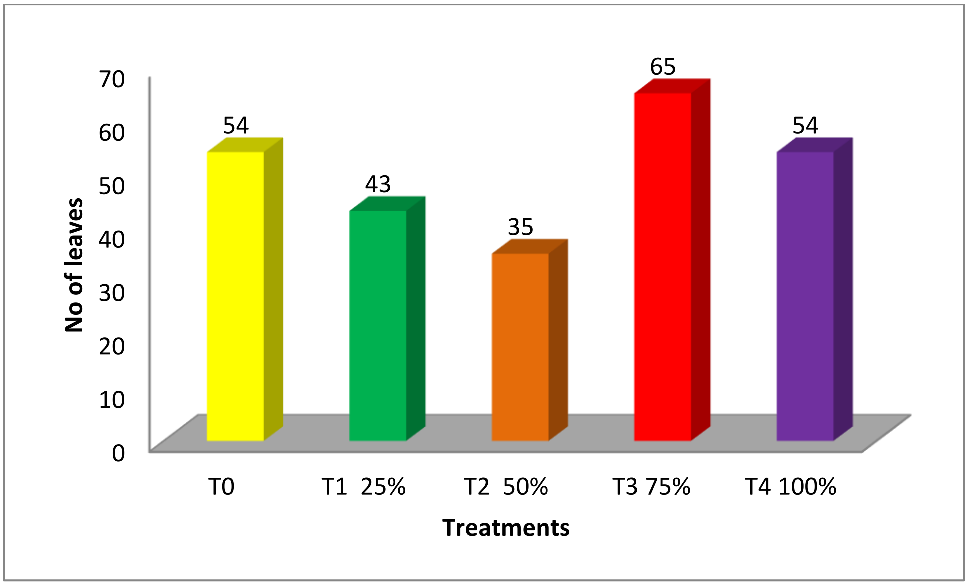
Plate 11

Growth of *Solanum lycopersicum* L. on the 75th day

Plate 12

Fruits on 75th day

Figure 10



Number of leaves

T0 – Control

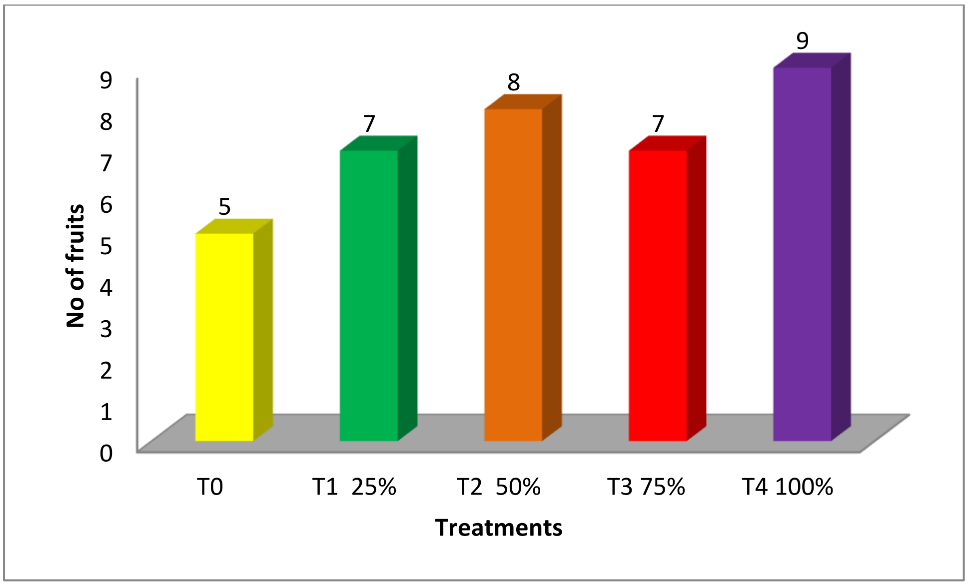
T1 – 25% of Papaya leaf extract

T2 – 50% of Papaya leaf extract

T3 – 75% of Papaya leaf extract

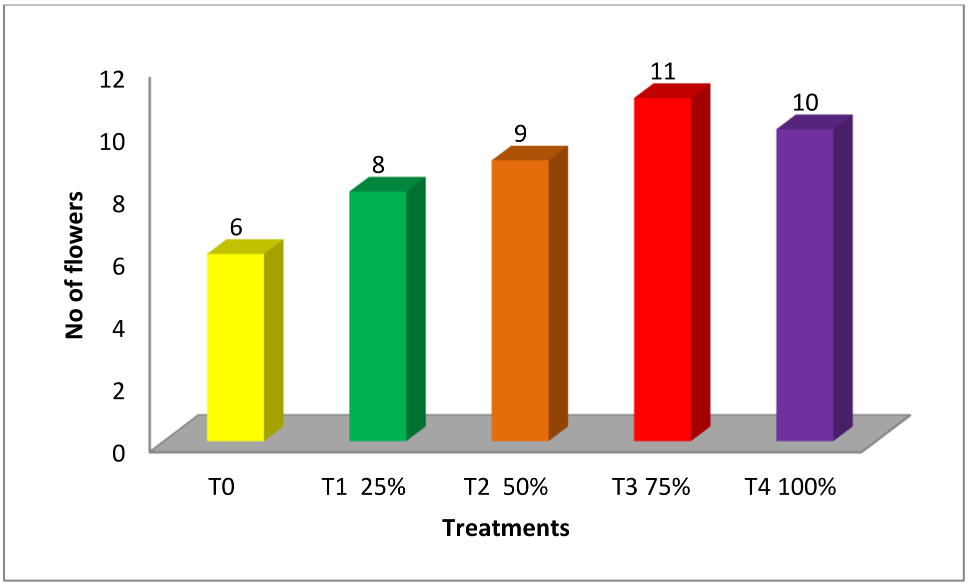
T4 – 100% of Papaya leaf extract

Figure 11



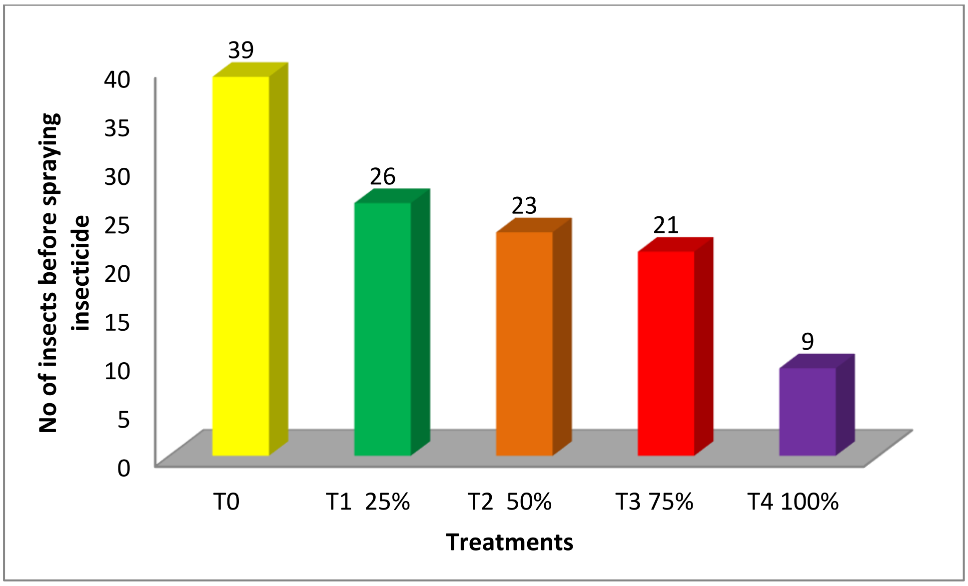
Number of fruits

Figure 12



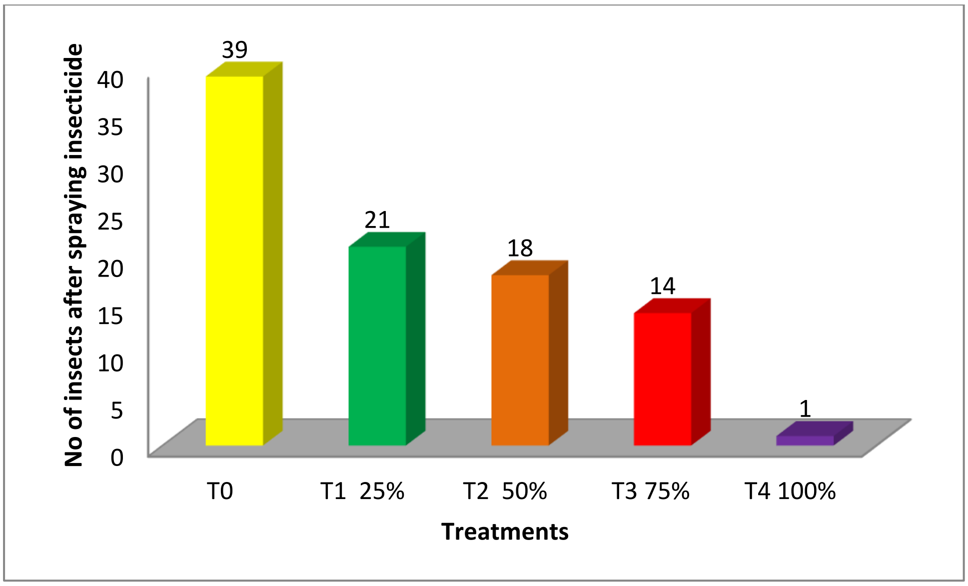
Number of flowers

Figure 13



Number of insects before spraying insecticides

Figure 14



Number of insects after spraying insecticides

The Number of insects before spraying insecticides were found to be 39 ± 4.8 (Figure 13) on the 75th day. After spraying insecticide it decreased in T_4 (100%) concentration to 1 ± 0.6 (Figure 14). The number of leaves, flowers and fruits on the 75th day were higher in T_3, T_4 and T_3 respectively. The measurement observed were 65 ±7.9 (Fig 10), 11± 0.6 (Fig 12) and 9 ± 0.3 (Fig 11).

Plate 13

Before spraying insecticides

Plate 14

After spraying insecticides

Plate 15

Flower of *Solanum lycopersicum* L.

Plate 16

Fruit of *solanum lycopersicum* L.

Papaya leaf extract has more effect on insect. The *Solanum lycopersicum* L. treated with 100% concentration of papaya leaf extract effectively controlled the insects on the 45^{th}, 60^{th} and 75^{th} day.

NUMBER OF DAYS	TREATMENT	RESULT	
		Before spraying	After spraying
45^{th}	T_4	6 ± 0.8 (Fig 3)	1 ± 0.3 (Fig 4)
60^{th}	T_4	32 ± 3.5 (Fig 8)	9 ± 0.6 (Fig 9)
75^{th}	T_4	39 ± 4.8 (Fig 13)	1 ± 0.6 (Fig 14)

Continuous spraying of bio-insecticide gives better result. Based on the result obtained, we can conclude that the use of papaya leaf extract responded significantly in the growth and yield of Tomato *(Solanum lycopersicum* L.). The treatment T_4 produced the highest mean value of 9±0.6 for the 1^{st} harvest.

The use of plant-based bio-insecticides in effectively controlling the insects could help in maintaining the fertility of the soil that in turn help in sustainable agriculture.

Efficacy test carried out by Wijanarko *et al*. (2017) have shown that papain extract was powerful to kill *Spodoptera litura*, especially in wet conditions

Oguh *et al*. (2019) have earlier shown that the botanical pesticides are highly bio-degradable and they become inactive within few hours, so the toxicity to humans is very low and are eco-friendly. In the current study, we have observed that the use of bio-insecticide using papaya leaf extract could reduce the insect pests in tomato plant and increase the yield of the crop.

The studies by Oguh *et al*. (2019) clearly indicates that the natural bio-pesticides breaks down faster and helps the soil to regain their healthy state within a short period. So, the use of naturally produced bio-insecticide and bio-pesticide could help in sustainable agricultural production.

Earlier studies carried out by Luca Ruiu (2018) have reported that the leaves of *Gliricidia sepium* could be used as a potential bio-insecticide for papaya mealy bugs.

Studies by Malabadi *et al*. (2017) have shown that the use of papaya leaf extract could be an alternative herbal medicine for dengue fever in the management of thrombocytopenia.

The current study on different concentrations of bio-insecticide obtained from papaya leaf extract on the growth of tomato plant is in accordance with the earlier studies by Idris *et al*. (2018) on the response of tomato to different rates of insecticidal spray on the growth and yield.

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted in Tomato plant in grow bags under control and four different concentrations of Papaya leaf extract. The seedling formation after germination started on the 10^{th} day of growth. The parameters tested for morphological characters were number of leaves, number of insects before spraying and number of insects after spraying the insecticide on the 45^{th}, 60^{th}, 75^{th} day after sowing. The reproductive characters such as number of buds and fruits formed were estimated for the study in control and treated plants.

On the 45^{th} day, number of leaves and number of fruits was found to significantly increase in plants treated with 100% papaya leaf extract as a result of decrease in the number of insects.

On the 60^{th} and 75^{th} DAS, the growth parameters and reproductive characters significantly increased and the number of insects also reduced drastically which shows that he bio-insecticide prepared from papaya leaf has a positive effect on the control of insects at 100% concentration.

Goodness of Bio insecticides

Bio insecticides are the natural insecticides produced from plants. Papaya plant extracts have the most effective compounds to eradicate the insects. They are cheaper and eco-friendly. The plant is easily available in all regions. It is safer than any other insecticides. Mainly it kills the targeted organism. Synthetic insecticides are more harmful, and create pollution. Bio insecticides, on the other hand, do not persist long in the environment and have shorter shelf lives; they are effective in small quantities, safer to humans and animals compared to synthetic insecticides; they are very specific, often affecting only a single species of insects and have a very specific mode of action; slow in action and the timing of their application is relatively critical.

Carica papaya is a plant that possesses multiple usages. Many researchers have carried out studies on the ethno-medicinal value of this plant. Leaves of this plant are widely used for various purposes. Bio-pesticides and Bio-insecticides are natural plant products that have a potential role in future Integrated Pest Management (IPM) strategy and also a significant role in agriculture and forestry.

Traditional farming system is an important tool to promote food and nutritional security for enhancing agricultural growth. More chemicals are used by farmers to feed the ever growing population. Pest induced loss in the yield of crops have paved way in formulating more reliable, sustainable and environment friendly bio-insecticides and bio-pesticides. Due to this demand and the effort by Government to mitigate climate change, the use of natural insecticides and pesticides are going to play a major role in future Integrated Pest Management Programme.

The current study is carried out only in grow bags and is only a basic research on the use of Bio insecticide and further studies are required to prove the efficiency of the papaya leaf extract under field conditions.

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