2

NEEM OIL AS BIOPESTICIDES

Muhammad Haziq Izahudin, Muhammad Hilman Rosmi, Noor Akhmazillah Mohd Fauzi

Faculty of Engineering Technology,
Universiti Tun Hussein Onn Malaysia,
Pagoh Higher Education Hub,
KM 1, Jalan Panchor,
84600 Muar, Johor, Malaysia.

Abstract*—Agricultural biotechnology is a wide logical method used to improve plants, animals, and microorganisms. The biotechnology cycle in the agricultural segment incorporates improving the harvests, living things, and microorganism's quality and resistibility by hereditary adjustment. Other than that, biotechnology in agriculture additionally known from the utilization of more secure natural sources to create an item that will be ready to ensure and build the creation of yields and domesticated animals, for example, manure and biopesticides. The World Health Organization (WHO) likewise had reported that the synthetic based pesticides would arrive at their constraints of utilization as it loses their adequacy after some time. This can be shown by the disclosure of pesticides obstruction bugs. The neem plant was accounted for as the top rundown spices that can possibly go about as a biopesticide; this is because of the presence of various dynamic mixes known as limonoids. The significant compound is the azadirachtin which assumes an essential job as bug sprays, and it executes the focused-on bugs by troubling their development and generative system. The neem oil biopesticides additionally are less harmful towards living beings and less contamination toward nature. In this way, the neem removed biopesticides are presently popularized and have been sold and utilized in different nations.

***Keywords*—** *Agricultural biotechnology, Neem oil, Biopesticides, Harvesting improvements, Resistance.*

2.1 Introduction

Biotechnology can be referred to as any technological application that uses biological systems, living organisms or their derivatives to generate or alter products or processes for a particular purpose. It is also a field of applied biology involving the use of living organisms and bioprocesses in technology, engineering, medicine and other fields that result in the development of useful by-products. There were various tools offered in biotechnology like fermentation, microbial inoculation, cell or tissue culture, enzyme technologies, embryo transfer, protoplast fusions, polyclonal or monoclonal antibody technology and recombinant DNA technology. It was reported that environmental protection and sustainable development for agriculture, natural resources, health and pharmaceuticals industries can be achieved when integrating the tools with other technologies [1]. Industrial biotechnology has been applied for a long time in a variety of fields including health care, food and fine chemistry. At present, this

technology is gradually approaching areas such as bulk chemistry and energy supply, in a region where sustainable development is the main keyword. In addition, the implementation and development of the biotechnology industry is mainly driven by a fundamental force, which is market economy. This occurs as biotechnology promises highly efficient processes at a lower operating and capital expenditures [2]. This kind of industry has a big capability to enhance and expand more in the future with the aid of development in research and technology. Furthermore, political and societal demands for sustainability and environmentally sustainable industrial structures, combined with the depletion of crude oil reserves and increasing world demand for raw materials and energy, will continue to push this trend forward [3]. Biotechnology applications nowadays are held in various areas such as in medical, industrial and agricultural.

Agricultural biotechnology is basically a wide scientific technique used to improve plants, animals and microorganisms. These techniques were studied and developed by the scientist for years in order to create various solutions regarding the problems from the increasing agricultural productivity. Biotechnology process in the agricultural sector includes improving the crops, animals and microorganism's quality and resistibility by genetic modification. Other than that, biotechnology in agriculture is also known from the use of safer organic resources to produce a product which will be able to protect and increase the production of crops and livestock such as fertilizer and biopesticides. These two products are very popular in substituting the conventional solution which is costlier and harmful for either the farmers or the surrounding ecosystem. As the main purpose of biotechnology is to produce cheap, safer and eco-friendly solutions, more research on developing a new solution has been made and applied to the agricultural sector. In agricultural biotechnology, there is a technique called organic farming which is a cultural crop production that involves the use of cultural and biological methods to enhance soil fertility and achieve ecological balance in the farming system [4].

It is a farming system that uses environmentally friendly methods of weed, pest, and disease control. It prohibits the use of synthetic pesticides and fertilizers, emphasizes animal health in livestock, preserves the overall harmony of the agro-environmental system and its biological diversity, and gives priority to renewable energy and the recycling of raw materials [5]. From an environmental point of view, it is a preferred method for pest and disease control in agricultural areas. Insecticides tend to pose a threat to agriculture, causing major losses in yields every year. One of the key approaches to overcome crop losses is the use of organic pesticides within the agricultural system [4], [6],[7].

2.1.1 Biotechnology on Biopesticide

Biopesticides are biochemical pesticides that are naturally occurring compounds that control pests by means of non-toxic mechanisms. Biopesticides also are living organisms or their products or by-products that can be used to counter pests that are injurious to plants. Biopesticides comes as a solution, since their uses lowers the risk of exposure to contaminants, decreases water contamination by fertilizer runoff, reduces the number of applications, causes less harm to beneficial pests, biodegradables and enhances nutritional quality [8].

Basically, there are three major classes of biopesticide that are microbial pesticides, biochemical or herbal pesticide and plant-incorporated protectants (PIPs). These biopesticides were grouped into three based on the main component of the product. Microbial pesticides are a type of pesticide that were created using microorganisms such as bacteria, viruses and fungi. This type of pesticide targets a specific species of pest in the targeted area. The microbial toxin is derived from microorganisms which can cause pathogenic effects on the targeted pests. The toxin microbial or also known as entomopathogen attacks the skin and multiply causing the host in this case, the insects die. Microbial pesticide is an alternative to the chemical pesticide which provides more ecological safety and specification of the targeted pest. Microbial pesticide has a better efficiency and offered more safety for human and other non-targeted creatures that lived in the surrounding ecosystem hence leading to preservation of other natural enemies of the pests [8]. Examples of microbial pesticide is the use of Bacillus thuringiensis to kill Aedes aegypti sp and use of specific fungi to remove certain weeds of the crops.

Next, biochemical or herbal pesticides are being produced by the naturally occurring substance such as plant extract, fatty acid and pheromone that helps to control the pests by use of nontoxic mechanisms. Biochemical pesticides have a unique way of controlling the pest which is interfering with growth and mating of the targeted pests instead of killing them or making them inactive [8]. The example of biochemical pesticides are canola oil and neem oil which have antiferedant

properties and deterring the growth and reproduction process of the pests.

Lastly plant incorporated protectants (PIPs) produced from plant genetic material that have been incorporated to the plant. The information of genes from the plants that have ability to resist the pest on its own. In the other words, PIPs are a mutagenic prose that have been introduced to the plant in order to produce substance to kill the pest. Example of PIPs is the gene of Bt pesticidal protein that has been taken to be introduced into the plant genome. The plants that receive the gene also have the ability of Bt bacterium sp to produce substances that can kill the pest [8],[9].

Figure 1: Neem leaves used to produce biopesticide.

In agricultural biotechnology applications nowadays, attention is increasingly given to the utilization of natural compounds (such as essential oils) as a viable alternative to agrochemicals in agricultural pest control. These aromatic compounds are derived from various aromatic plants, which are rich sources of biologically active secondary metabolites like alkaloids, phenolic matter, and terpenoids [9]. The application of natural compounds as alternatives to pesticides can refer to the application of Neem plant oil as biopesticides. Neem is one of the most popular biopesticides for botanical sources (Figure 1). The neem plant has been known for its effectiveness against insect pests for three decades. Leaves and seed extracts of neem plants have been reported for their deleterious effects on insects. Furthermore, the neem's oil is extracted from the neem seeds and leaves in which these parts of the tree contain much of the limonoid, azadirachtin that is evaluated as the most effective botanical pesticide used to counter more than 400 species of insects. It also can be easily grown in a nutrient-poor soil and can survive even in harsh conditions. It is considered to be nice with other plants, but harmful to insects [10]. As a biopesticide, azadirachtin is considered to have very little effect on non-target species, such as pollinators. It is absolutely non-toxic to vertebrates.

Azadirachtin may not be effective against all pest insects and its effectiveness depends on the dosage used (Figure 2). According to its capabilities, it is selected to be used as biopesticide and replacing the use of synthetic pesticides in agricultural production. Table 1 shows the applications of neem trees act as biopesticides with the use of nanocarriers for the agricultural sector. Neem trees show the ability to fight and eliminate various pests and insects. This product has its own characteristics that are able to resist the pests by controlling the growth and reproduction, antifeedant effect and toxicity properties of the compound.

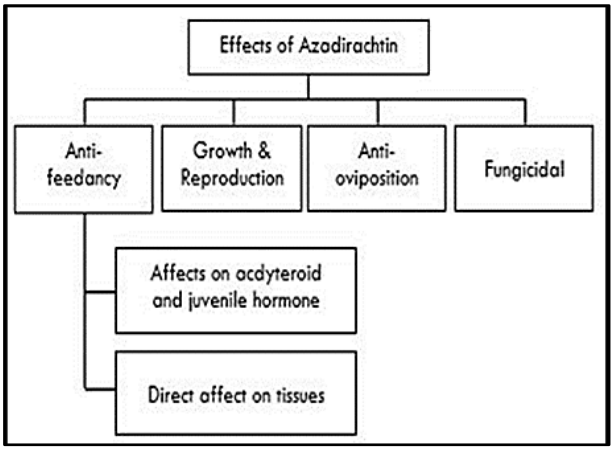

Figure 2: The distinct effects of azadirachtin [10].

Common conventional pesticides nowadays have been widely used in the agricultural sector which is to be sprayed on the crops or the storage to protect them from being damaged by pests and insects. Conventional pesticide consists of active substances such as chlorine, oxygen, sulphur, phosphorus, nitrogen and bromine which means to kill and exterminate the pests and insects. However, these products containing high concentrations of chemicals are basically genotoxic and cytotoxic which can cause harm to the farmer working on the crops and other living creatures. Conventional pesticide can cause damage to the genetic information within cells which can cause mutation and lastly may lead to cancer. It also can cause damage to the living cell inside the body system when being exposed to the chemical for a long time during the treatment process.

The properties of the pesticide which takes a longer time to be neutral and excessive residue from the treatment process could also cause harm

to the environment and may lead to the ecosystem disturbance. Biotechnology engineering introduced biopesticides which have been manufactured using derived nature materials such as animals, plants and bacteria which have their specific gene and ability to control and eliminate the pests. The material used to produce biopesticide is safer and low cost compared to the conventional pesticides [11]. Biopesticides also is an environmentally friendly product as it reduces the harms to the other living creatures in the area, does not leave harmful residues and is specific to targeted insects due to their biocontrol agents that are contained in the nature materials. One of the derived compounds to produce the biopesticide is by using azadirachtin indica which is also known as neem trees. Researchers found that the leaves and seeds of the neem tree were able to produce enzymes which could help to fight the pest and insects. Neem was chosen because its compounds have antifeedant effects and the ability to disturb the insects' hormone besides the residues of this substance does not harm the consumers making it a unique pest control compound [12]. Neem is also less harmful to the mammals and can combine well with chemical, botanical and microbial pesticide [13]. However, the toxicity of the extract depends on the ways it was extracted. Neem leaves that have been extracted at non-aqueous appear have a high toxicity compared to the aqueous extract. Tests conducted on the mice for the aqueous shows that there is no cytotoxic effect compared to the non-aqueous extract which can cause allergies to humans and bring negative effect to human reproductive ability at high concentration [12]. From the data obtained it is sure that neem plant can become a great biopesticide with a lower probability of damage to humans and the environment and higher mortality rate to the selected insects.

Table 1: List of nanocarriers used to encapsulate neem active components [12].

Component	Active ingredient	Carrier	Application
Neem	Azadirachta	Carboxymethyl chitosan with R-CM-chitosan	Botanical pesticide
Seed Kernels	Azadirachta	Nanoemulsion	High mortality against a storage pest
Neem oil	Azadirachta	β -cyclodextrin and PCL	High efficiency against nymph and eggs of Bemisia tabaci
Neem extract	Azadirachta	PCL	Exhibit 100% larval mortality against Plutella xylostella
Neem oil	Azadirachta	Silica NPs	Exhibit significant insecticidal effect against Tuta absoluta
Neem leaves	Azadirachta	Silica NPs	Exhibit strong anti-fungal properties

A Azadirachtin Structure and Effect

Azadirachtin is a compound with molecular formula of $C_{35}H_{44}O_{16}$ which is also known chemically as tetranorterpenoid (Figure3)[10]. The structure of this component is also been elucidated and being proved to be effective against various insect species including Orthoptera sp (grasshopper), Homopter asp (leafhopper), Dictyoptera sp (cockroaches), Lepidoptera sp (moths and butterflies), Heteroptera sp (true bugs), Diptera sp (flies), Coleoptera sp (beetles), Hymenoptera sp (bees and wasps), Isoptera sp (termites), Thysanoptera sp (thrips) and Siphonaptera sp (flea) [14]. Those are the pest species that always cause damage to the crops as they consume the plants to live. Azadirachtin also has a specific characteristic which affects the hormone of the insects and eventually stops them from breeding and reproducing.

Breeding and reproducing process is very important for the insects as with these systems being interrupted, the population of the pests will be facing a huge reduction and may eliminate the whole colony from the area.

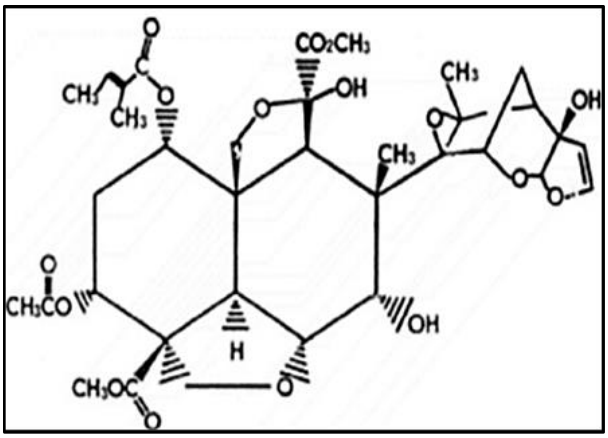

Figure 3: Chemical structure of azadirachtin [10].

Azadirachtin is highly reactive and has affected both the cytoplasm and the nucleus of the insects' cells. It also acts on mitotic cells which block the insect's life cycle because of its anti-proliferating effect. It causes damage to the DNA and binds them to a large protein complex which is identified by ecdysone receptors that intrude the development of the insects [10], [12], [13]. The insect's development and growth are also dependent on their endocrine and neuroendocrine systems. These systems are very important to the insects and pests to regulate the development process hence the application of azadirachtin on the plants will cause adverse development effects. The effects of the azadirachtin application will reduce the population of the unwanted insects to inhibit the targeted area [14].

Azadirachtin also has some other characteristics which helps to eliminate the pest which can cause oviposition deterrence on the pest. This compound prevented the females from depositing eggs [15]. Azadirachtin is able to reach moderate oviposition deterrence which is around 22.28% on the fruit flies [16]. When biopesticide containing azadirachtin was being applied to the crops plant, making it to be not a suitable host for the pest to lay their eggs. This is because the pest no longer recognizes that host as their preferred oviposition site because of the altered chemical caused by the azadirachtin [17]. Moreover, neem-based pesticide also blocks the neurosecretory cells which can cause disruption on adult maturation and egg production [15]. As the female pest does not even have chance to lay the eggs and the retarded maturity of the adult pest caused the amount of them inhibits the crops area will be decreased and eventually the pest will move to the other host plant.

Azadirachtin also have antifeedant effect to the pests and insects which with the presence of this compound, insects and pests does not feed on the leaves and surfaces of the crops [15]. Azadirachtin able to disturb the feeding stimulant receptor of the herbivore pest which is by blocking and reducing the sensitivity of sugar sensing cells. With the aid of this compound, the insect will not be able to assess the nutritional adequacy correctly which will lead to death due to insufficient or excessive food consuming [17], [18]. Azadirachtin also can cause a reduction in the post injective digestive efficiency which is also known as "secondary" antifeedancy. This compound causes disturbance to the hormonal as well as physiological systems which cause hindrance in the food movement through the insects' midgut and inhibition in production of digestive enzymes [19], [20]. In an easy term, the insects will have problems digesting the food which may cause them unable to process their food and die due to lack of nutrients for their body. Since high concentration of this compound could cause harm to the beneficially insects, a product with low concentration of azadirachtin is more preferred at least in the aid to providing crop protection by reducing the pest population without harming any other beneficial insects or natural predator population[21], [22].

Next, neem products are able to interfere with the pest's growth regulation where this product attacks on the juvenile hormones [23], [24]. Azadirachtin also interferes with the growth and molting process of the insects. Antifeedant properties on it cause a serious problem to their ingestion systems which could lead to abnormal smolts and growth reduction. The insect's larva basically will shed off their skin as they grow bigger and will be governed by ecdycone enzymes. As the neem compound accesses the body of the larvae, ecdysone activity will be suppressed which causes the larvae to fail to moly and lastly cause death[25],[26],[27]. Indirectly this compound affects the neurosecretory system in insects which could cause inability to translate the neural signal in their body and next cause failure to undertake the process of the body[28],[29]. Azadirachtin blocks the discharge of morphogenetic peptide hormones like prothoracicotropic hormones that control the prothoracic glands and allatostatins. This condition will next take control of the corpora allate which is responsible for secreting juvenile hormones for the insects [30],[31]. Molting hormones from prothoracic glands are responsible for controlling the formation of new cuticles and play a central role in ecdysis. The formation of juvenile stages during each molt is controlled by the juvenile hormone from

the corpora allata [32],[33]. As the growth rate of the insects have been controlled and delayed, the population of the targeted pests and insects on the agricultural area can be reduced and the protection of the plant can be achieved. The competition between the beneficial insects and the pest can be reduced, hence bringing a lot of benefits to the area and increasing the crops yield.

B Neem Derived Compounds

Besides azadirachtin, neem also contained other compounds with pest resistance characteristics which also helped to eliminate the insects and pests. One of the compounds is saponins which is also known as surface active glycosides as it is easy to dissolve in water and form a foaming solution (Figure 4) [19]. Saponins made up from aglycone connect with the hydrocarbon chains. Saponins were proved to have antimicrobial activity and also could protect the plants from the insects [10]. Research shows that saponins are able to interfere with the pest's feeding behaviour and growth regulation. This compound can lengthen the larvae stages, delay the maximum size of larvae stage, causing ecdysial failure and reduction in the adult emergence [19]. Researchers also found that some saponins are also toxic to certain insects which cause disturbance to their growth stages. As for the microbial activity, saponins are recorded with antibacterial and antifungal properties which could help the plants fight pathogens [20].

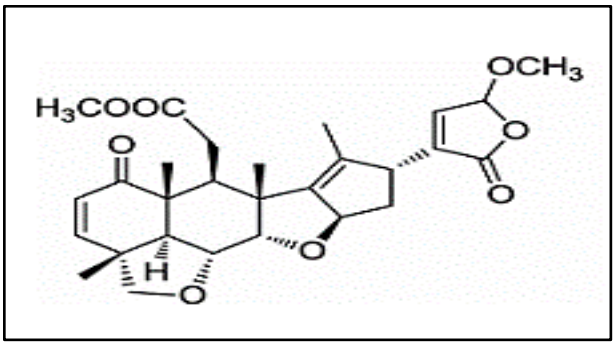

Figure 4: Saponins structure [19].

Azatin is also another compound in the neem that has antifeedant activity to the insects. Low concentration of azatin could reduce the growth rate of the pest while higher concentration could bring mortality to them. Azatin acts as a direct toxicant which makes larvae unable to feed when being exposed to it [10]. Research showed that azatin and nimbin (Figure 5) have been applied to the crops causing feeding deterrence and increasing the mortality of the larvae [21]. Around 80% to 90% of larvae mortality shows that azurin and nimbin as a successful product in pest controlling industry.

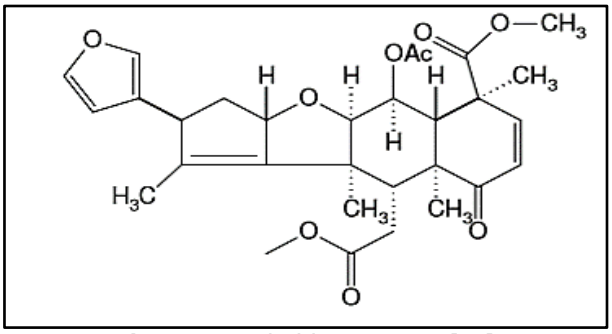

Figure 5: Nimbin structure [21].

Next, another compound that can be obtained from the neem trees is salannin (Figure 6). Salannin that has molecular formula of $C_3H_{44}O_9$ is also known as Azadiractin Margosan-O is an active component of neem with the ability of insects' growth regulating and antifeedant activity. Salannin have almost the same function as azadirachtin which it deters the insects' feeding, increases the larval stage duration and delays the molt process of the insects causing the reduction of unwanted pest population due to the low birth rate and high mortality [32].

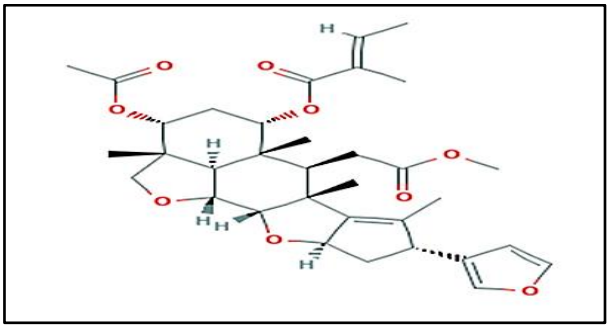

Figure.6: Salannin Structure [32].

One research has been conducted on investigating the biological activities of salannins compound from azadirachtin indica sp on the two types of insects that are Helicoverpa armigera and Tobacco armyworm Spodoptera litura sp. The salannin compounds used were 3-O-acetyl salannol, salannol and salannin and the results for all their samples caused deterrence on feeding and reduced the growth rate on the larvae [34].

C Neem Oil Preparation

Leaves and seeds of the neem plant must be collected and prepared before starting the

extraction process. Several steps have been taken to ease the extraction process according to the Prem Baboo method. Two ways have been introduced in order to obtain the azadirachtin from the neem leaves and seeds: that is by crushing the seeds into powder for the extraction process or squeezing the seeds to pull out the oil from the seeds.

The neem oil preparation is illustrated in Figure 7. The kernels of the neem plants were collected from the garden without dirt and sand so that the next process will not be disturbed by them. The seeds and leaves were cleaned before being crushed. Crushing process is being conducted to produce powder for the extraction process and pull out the oil from the seeds. There are two methods on extracting the oil from the seeds that are mechanical press method and steam pressure extraction. Mechanical press method used hydraulic pressing equipment to introduce the pressure to the seed until the oil is pressed out of it. Then, a filtration process is being conducted to remove other unwanted particles in order to obtain a pure neem oil. However, steam pressure extraction is being conducted at a high pressure with the help of the steam. The seeds were heated by steam which caused the oil to flow out of the seed, it is easier as the seed becomes swollen and squeezing part to extract the oil becomes easier [32].

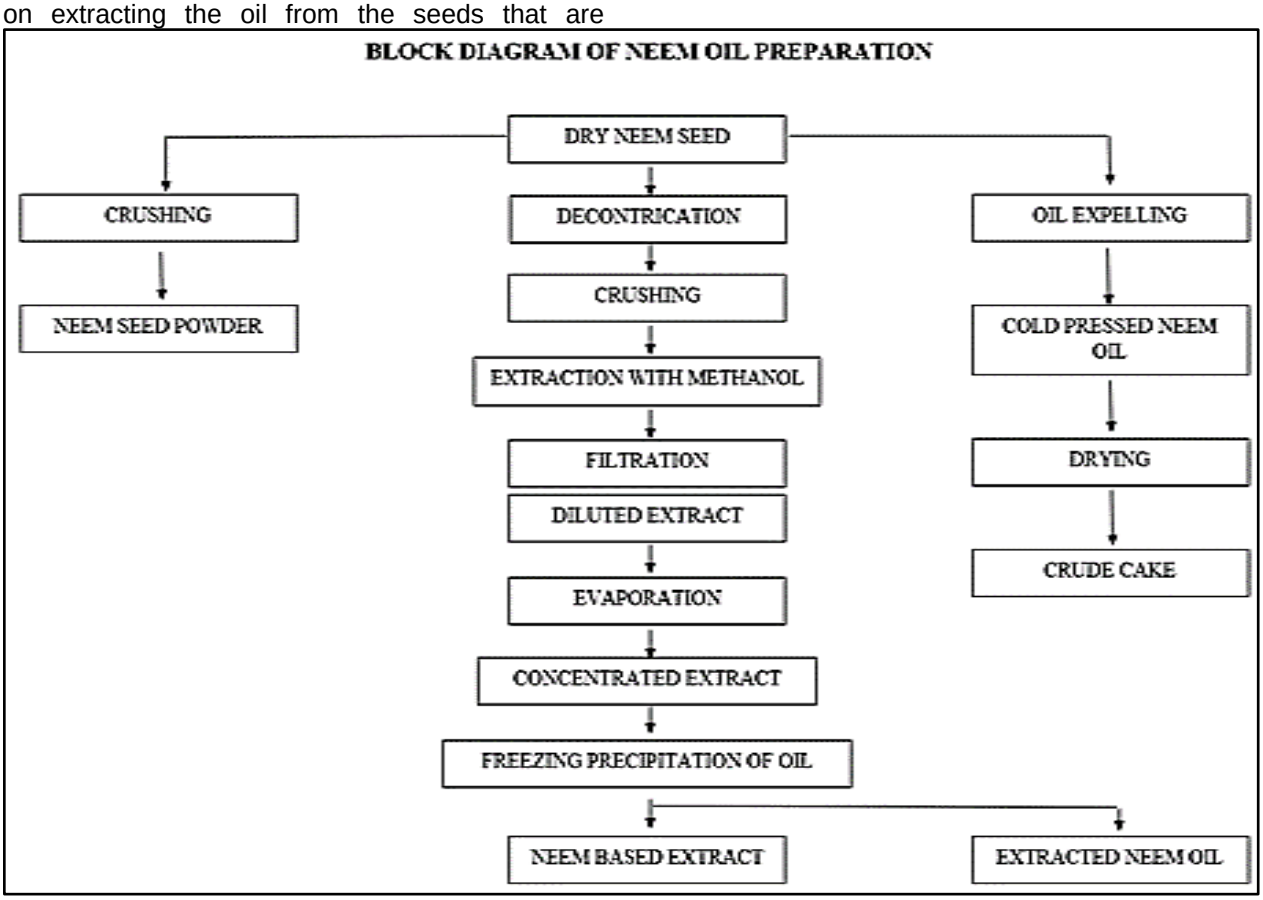

Figure 7: Block diagram of neem oil preparation [23].

D Extraction of Azadirachtin indica (Neem) Leaves

The neem leaf is being extracted to obtain the toxic property needed to eliminate the insects and pests on the crops. The surface of the leaves was sterilized using 1.0% sodium hypochlorite to make sure that only the required product was being obtained. The leaves are then grounded in a mortar and was transferred into a beaker. 10 ml of distilled water was being added to the beaker then being covered with sterile aluminum foil for four hours to make sure that neem extracts are completely diluted in the water without any contamination from the air such as dust. As for

the neem seeds, the same procedure has been applied but 15 ml of distilled water was being used instead of 10 ml for neem leaves. The mixture is then being filtered to remove the pounded leaves [22].

Extraction with alcohol also can be done and bring a better result [23]. From the method proposed by previous study done by Silva (2012), the neem leaves were first dehydrated in the oven at 40℃ for about 96 hours. The leaves were then being pounded into powder by using the knife mill. The powder then was being put into an extractor Soxhlet with 300 ml of solvent. Three types of solvents were being used to make sure that the complete extraction can be obtained at the end of the process. First solvent used was hexane and the sample was being left for 24 hours of reflux. Then hexane was being removed and dichloromethane was added and left for 12 hours of reflux. Lastly, a second solvent was removed, and methane was added and left for reflux for 13 hours. These steps help to make sure that the extraction was completed, and the solvent becomes colourless after long exposure of the product which verifies the completion of extraction. Using a water column at low pressure and 40℃ temperature, the extracts then were concentrated in a rotary evaporator and lastly the extract was placed in a glass flask in the laminar flow cabinet until the solvents completely evaporated [23].

Next, according to Prem baboo's extraction method, it only uses one alcohol solvent for the whole process. The seeds were manually cleaned thoroughly from leaves and dirt by the air blower before applying hot air to the seeds until 55-60 ℃ to remove the moisture. Then preparation of the seed was done before starting the extraction process. The researcher conducted the process by using five types of solvent for five different Soxhlet extractor that were coupled with a heating mantle. The solvents used are ethanol, hexane, 60% ethanol with 40% hexane solvent, 60% hexane with 40% hexane solvent and ethanol hexane equimolar solvent. Five types of solvent were being used to identify the solvent that was able to extract the most oil from the neem seeds since the aim of biotechnology is to reduce the waste and cost of the production [32].

Table 2.2: Properties of neem oil extracted [32].

Solvent	Acid value	Sap Value	Specific gravity	pH
Ethanol	19.35	198.5	0.87	5.7
Hexane	20.05	201.0	0.90	6.1
50/50 Hexane/Ethanol	18.79	198.7	0.91	5.9
60/40 Hexane/Ethanol	12.9	200.0	0.92	5.8
40/60 Hexane/Ethanol	17.11	198.4	0.90	5.8

From the table, hexane was proven to extract the most amount of oil from the seeds compared to the other four solvents. Hexane recorded 201 sap value which is the highest followed by 60% hexane with 40% ethanol that only recorded 200 sap value. Acid value of the hexane is also the highest, which is 20.05 which mean the extraction process with hexane solvent is very suitable becoming pesticide based on the ability to extract a large amount of oil with high acidity value that can be used to kill the pests and the unwanted insects. Besides, Prem baboo also conducted another extraction method known as supercritical extraction in which the process only uses carbon dioxide as the solvent at a supercritical point of the gas.Supercritical extraction at very high pressure and temperature where after the extraction process is complete, the carbon dioxide will completely be evaporated leaving no solvent residue. Only pure extraction substances will remain at the end of the process. The process started with the neem products being extracted right after the harvesting process to keep all healing phytochemicals with highly purity. The carbon dioxide pumped at a super critical point where the temperature and pressure are at the condition the gas becomes a liquid. The carbon dioxide pumped will rapidly diffuse into the solid matrix of neem product which then will dissolve the neem oil. The neem oil is then removed from the extraction cell into the separator and then being discharged for further uses [32].

2.2 Advantages of Neem Oil as Biopesticides Compared to Conventional Pesticides

Pesticides are any substance or chemical which is commonly used in agricultural production to avoid or manage pests, diseases, weeds and other plant pathogens, with the goal of minimizing or abolishing yield losses and having decent production quality [6]. In Indonesia, excessive use of pesticides caused significant environmental problems, such as acute and chronic human pesticide poisoning, animal poisoning, contamination of agricultural products, extinction of beneficial natural parasites and plant predators, and pesticide resistance in pests [7]. Thus, organic pesticides are used as substitutes to conventional pesticides.

Biopesticides with neem oil based was listed as one of the registered biopesticides used in the agricultural sector in India [8]. This plant extracted oil has been studied by many researchers for its capability to be used as biopesticide. Study shows that this plant contains at least 100 biological active compounds which include limonoid, azadirachtin as a major component which affects about 90% of most insects [9]. Synthetic pesticides are of significant concern because they accumulate in the environment and so much attention has been dedicated to natural compounds that may replace synthetic ones. In addition, excessive use of synthetic pesticides have been reported to cause pollution toward environment and health issues to the living organism. Thus, biopesticides are a good alternative to synthetic ones since they produce a limited amount of environmental waste, a low degree of toxicity to humans and many other advantages. Next, the advantages of using neem oil based as biopesticides could be discussed in terms of cost, production and energy, eco-toxicology and environmental and the difference compared with conventional pesticides.

A Advantages in Term of Cost

Neem oil-based pesticides is a natural product which is obtained from the extraction of seed and leaf of the Neem tree. It is said that pesticide is costly compared to biopesticides [24]. Biopesticides are classified as low cost compared to the synthetic pesticides as the cost needed to make it as biopesticides are cheaper compared to synthetic pesticides [25]. The synthetic pesticides which were merely chemical based required high-tech equipment and underwent complex processes to produce it. This causes an increase in energy equipment and chemical cost. Other than that, there is also additional cost applied for synthetic pesticide due to the waste management process [38].

In addition, this plant availability and capability makes it more cost effective as it is easier to plant and collect for extraction purposes. It is said that the neem tree is tolerant to most soil types, including dry, stony, lateral crusts, heavily leached sands and clay, including marginal and leached soils. The other treasure of neem is within the fruit, as it is produced in great quantities, and there are about 4000 clean seeds per kg of neem [26].

Furthermore, the waste from the extraction of neem seed, seed cake obtained during the process can be used or sold as natural fertilizer which can be used in common agricultural practices [27]. Neem leaves also can be part of biopesticide as it also has azadirachtin component which helps eliminate the pests and insects. The use of neem leaves shows that the same tree can be used to obtain the seeds and the leaves. Low production cost will be needed compared to synthetic pesticides which for every production of product, new chemicals need to be synthesized and produced. Thus, the use of neem oil as biopesticides proved its capability to substitute the conventional pesticides in terms of cost for obtaining raw materials and production process.

B Advantages in Term of Production Process and Energy Consumption

Production of pesticides is well known to require a range of methods and processes to produce the final product as it is based on a chemical substance. The pesticides are manufactured by complex chemical processes and have been carried out by a well-trained chemist. Besides, sophisticated laboratories are also needed in large capacity to produce a high-quality pesticide to suit the agricultural demand. Thus, the energy consumption is merely higher than the biopesticides production process.

In production of neem oil, the processes required are simpler compared to the production of synthetic pesticides. Two major steps are involved in the preparation of organic neem tree pesticides. The first step involves collecting, drying, roasting, crushing and pressing ripe seeds to extract neem oil. In the second step, the oil is then formulated into a pesticide by diluting it

with water to the required amount. The extraction of neem seeds is said to work on a basic mechanical system using a seed pressing machine that can be either adapted from a standard oil extraction machine or easily fabricated by local technicians using basic specifications [4].

The method used for neem oil extraction was uncomplicated and easy to develop in areas with limited technologies compared to conventional pesticide which required specific and more complicated technologies as it deals with chemicals [25]. Neem oil preparation method does not require high level training to run the production and methods used are easy to apply. This shows that neem oil biopesticides are more efficient compared with synthetic pesticides in terms of energy consumption in production and low specification level of technologies involved.

C Advantages in Term of Eco-Toxicology and Environmental Safety

Pesticides are generally made up of any substance or chemical that is being used in agricultural production for the purpose of killing harmful living things. It was also reported to cause pollution to the environment and being a threat towards health and safety of farm workers and also consumers. In addition, the Food and Agriculture Organization (FAO) had reported that the use of pesticides had produced an increasing number of perennial insect species that are immune to pesticides [6]. This was a sign in which conventional pesticides must be replaced with a better formulation or pesticides that are safer and eco-friendlier towards the environment and consumers.

On behalf of the growing awareness of the harmful side effects of chemically synthesized pesticides and insecticides, more and more focus is being placed on the use of biopesticides. Several countries have already prohibited or limited the use of large numbers of these synthetic chemicals that have been classified as highly toxic or hazardous [28]. The neem plant has been classified as the most reliable botanical source of biopesticides [29]. This is because neem plant is said as one of the least toxic to humans and shows very minimum toxicity towards beneficial organisms. There is also scientific research that has shown that neem is safe for workers, without risk management, and can be used during the entire crop production process and it also causes a little environmental pollution compared to synthetic pesticides [9][10]. The features of neem show that it is capable of contributing to a more sustainable organic agricultural production system that does not produce chemical residues.

Next, the neem oil does not leave residues in the environment as the azadirachtin are easily degraded in the environment and its half-life is short. The azadirachtin in neem oil can degrade easily when exposed to sunlight or ultraviolet rays, thus preventing their accumulation in the environment. Furthermore, it is rapidly breaking down by microbes and light breaks in soil, water and on plants with different time intervals. The half-life is up to 3 to 44 days in soil, 48 minutes to 4 days in water and 1 to 2.5 days in plant leaves [25]. According to the Environmental Protection Agencies (EPA) of the United States, they had issued a detailed and thorough study on the eco-toxicological effects of neem oil. The findings of the study are that there are no toxicity risks associated with the use of neem oil. EPA carefully studied the toxicity of neem products and concluded that: On the basis of existing evidence, there is no reason to presume that neem oil can affect the ecosystem, including beneficial insects such as bees. In addition, they also clarified that neem oil is not harmful to non-target organisms and will have no impact on any currently listed endangered species or any designated critical habitat on its usage pattern and use instructions [25]. Thus, the neem oil proved that it can be a good alternative in replacing pesticides for agricultural production as it is less toxic and more eco-friendly towards the environment compared with the pesticides.

2.3 Effect of Neem Oil as Biopesticide On Today Society

Nowadays, awareness about the effects of pesticides have been spread worldwide and the new approach towards more natural and less toxic pesticides are the main focus. Biopesticides have been accepted by society as they introduced a better result with minor side effects to the environment and human beings. Besides the manufacturing cost can be reduced, this product also ensures the safety of the other creatures in the surrounding area and also increases the economics of the country and the livestock yield of the crops. The society also gets a lot of benefits from the biopesticide as the ecological surrounding is not being disturbed and the safety of human beings exposed to this product have been ensured.

A Increase the Crops Yields

Main problem in the agriculture sector on production of food is the presence of insects and pests that cause damage to the crops thus causing limited production of the product. These pests continuously eat the leaves and fruits of the plants which caused damage and can no longer be used or consumed by humans. These insects that made the crops to eat their food will cause the product yield and quality decrease hence will cause profit loss for the agriculture sector.

Neem oil's biopesticide helps by eliminating the threat besides giving a better performance to the environment. Neem's compound, also known as azadirachtin, works to disturb the growth, metamorphosis and reproduction of over 400 insect species [14]. As the pests and insects have been eliminated, the safety of the corps has been secured hence increasing the quality and livestock yield of the crops [30]. On the other hand, securing the corps' safety also ensured the export revenues increased and reduced the international spread of disease from the other country as the food product imported into the country was reduced due to the sufficient resource of the product. This is because the import activities will be reduced so that only a small amount of fruits and other agricultural products will be taken into the country. Furthermore, there is a study that shows that the use of neem oil as biopesticides for Cowpea plants can produce a comparable yield compared with the conventional pesticides (Cymethoate) and control (borehole water). The researcher stated that it can be presumed that its potential for use as a control agent for cowpea insect pests was very great [36].

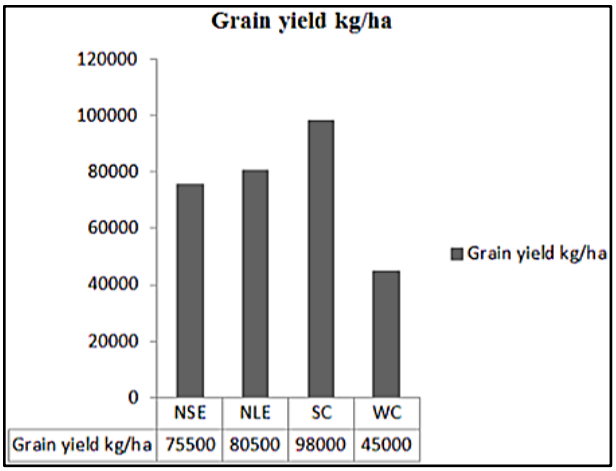

Figure 8: The effects of different pest control methods in yields [36].

In the **Figure 8**, we can see that the treatment which uses neem extraction as pesticides can provide a good result towards the yields. The NSE (neem seed extraction) and NLE (neem leaves extraction) was used in this study to compare its effectiveness with the synthetic pesticides in terms of yield. The graph shows that by using synthetic chemicals (cymethoate) can produce up to 98000 grain yield kg/ha and the use of NLE can come out with 80500 grain yield kg/ha. This result proved that the implementation of biopesticides which we take here NLE for example can produce a comparable yield with the use of synthetic pesticide. Even though the synthetic pesticide can produce much higher yields, it should be remembered that this kind of pesticides has been proven to cause many problems towards living organism's health and environmental issues. Thus, biopesticides are the right choice for a more sustainable and green approach towards a better health and environment. In consequence, with high crops yields in agricultural production helps in improving incomes rate for the farmers and also give a good impact toward the economy of the country.

B Safe for The Consumer Use and Their Environment

Chemical or synthetic pesticides bring harmful effects to the human and also the environment especially to aquatic lives. The pesticide residue that has been used in the agriculture sector flowed into the river causing the aquatic lives to live with it. It is very important to make sure that the product being applied in the sector is more environmentally friendly and has less side effects to the environment. This is because the effect may cause disruption to the other living creature and even cause death to them. The ecosystems will then be unbalanced and may destroy the environment.

Neem oil does not cause non-specific toxicity to mammals and humans and only attacks the targeted pest [16]. The ability of the product is to attack only targeted pests and this reduced the probability of the other living organisms to get affected by the product This biopesticides also was designed to not give any effect toward beneficial insects as this group of insects are needed in pollination and pest control processes. In addition, agricultural production that uses neem oil based as biopesticides not only promotes a high-quality product in production, but the product also is safe to be consumed by

consumers. Furthermore, there is a research carried out which reported that there are no major adverse effects toward exposure to neem oil for adults and children and no sign of skin irritation toward house pets [28]. The low toxicity effect of neem oil toward humans and environment and its eco-friendly characteristics hence improve safety for the consumers and other animals.

C Production of Safer Livestock

Chemical and synthesized pesticides have recorded a great result in managing and eliminating the pest in the agriculture sector; however, this product is ecologically unacceptable due to the damage taken by the environment, especially to aquatic life [15]. Moreover, based on early US data on 1990 shows that pesticides ranked 28 on number of deaths per year from 30 of other hazards to consumers [30]. Although the rank was very low, death upon using the pesticide also means that the toxicity of it could bring harm to human beings. The toxic could harm workers on the crops as they are the one responsible to apply the pesticide to the plant.

However, with the application of biopesticide made of neem leaves ensure the safety of workers from exposure to the toxic substance. This is because the neem biopesticide is very easy to use and safe. Workers will not be affected when the products that have been applied on the leaves or any part of the plant are dried. The substance only dangerous during treatment is being conducted where the workers and farmers are required to put on their protective equipment during the process. As the product is not harmful when dried, the substance residue is easy to be handled. The product will be solidified when being under 50 F temperature and introducing the product to the heat will cause the active ingredient in the oil to be destroyed [31], [35]. Furthermore, the applied biopesticides are also easily dried and becoming less so that the crops that are being harvested will not cause any side-effects to the consumer. Compared to the use of conventional pesticides, the toxic substance will be deposited on the harvested crops and need additional process to clean it off before it can be consumed. Thus, neem biopesticides help in production of safer foods to the consumer.

Besides that, the neem oil residues have a short half-life when they are accumulated in the environments [25]. Thus, the residue will not cause any pollution and side effects towards other living organisms. This short half-life was a good property compared to the conventional pesticides as they are deposited or accumulated for a long period of time in the environment which causes pollution and health problems to the living organisms.

D Job Opportunity for Society

The World Health Organization (WHO) has already called for an immediate ban on the use of dangerous synthetic pesticides that cause serious problems in the skin, kidneys and liver. Other than that, the government in India and Europe has already banned the use of a range of highly toxic and dangerous pesticides and has placed limits on the use of many others to avoid environmental pollution. The widespread response to synthetic insecticides and the need for environmentally sustainable and effective alternative pest control for agriculture have led pest control experts to turn their attention to plants as pesticides sources [28]. Furthermore, according to advantages offered by biopesticides from natural sources which are low cost, less toxicity towards human and animals, sustainable, degradable and high availability as raw materials enhance worldwide attention and results in a surge of commercial interest.

This attention was a good sign for the development of biotechnology in the agricultural sector for a better agricultural production. The utilization of biopesticides from natural resources would probably increase job opportunities for the society to make it as an alternative for replacing the use of conventional pesticides and to commercialize it. The job opportunity is offered to the society such as in the research and development sector of biotechnology, production sector for biopesticides and plantation of raw materials[36]. In addition, the production of natural biopesticides, for example neem oil, are more cost effective compared with the conventional pesticides as it is only required by a low specification of machines to extract the neem oil. Besides, countries with low availability in specified and high-tech machines also can have carried out the production of these biopesticides. It was reported that the Gambia which is a small west African country are able to use neem oil biopesticides. In addition, the neem oil was extracted by them using simple and low -tech machines which are said to be easier to be built by any technician [37], [38]. Furthermore, it is said that the waste obtained from the extraction process of neem seeds and leaves have a

potential to be marketed as bio-fertilizer. This is an opportunity for the society to begin a new business for a greener fertilizer in agricultural sectors, hence offering a lot more chances for the community to get a job. The waste can be used as a source of raw materials for a new company to run their business as by doing this, they can achieve the goals of industrial ecology. Other than that, this action makes the process of neem extraction to be more sustainable.

CONCLUSION

The modern agricultural pesticides which mostly are chemical based are not really a new thing to the world nowadays. This kind of pesticides are well known for their capabilities and effectiveness in increasing the production yields and to kill pests and insects. Over a period of time, being implemented by most farmers in different levels, it is probably bringing a lot of problems towards the environment and health of living organisms. There are various studies that show that the use of pesticides are harmful not only to the pests but also to the other living organisms and the surrounding. It is also not designed to kill specified groups or types of targeted pests; thus, the beneficial insects were affected, hence bringing problems to the natural process such as pollination. The World Health Organization (WHO) also had announced that the chemical-based pesticides would reach their limits of use as they lose their effectiveness over time. This can be shown by the discovery of pesticides resistant insects.

The problems encountered from the use of pesticides had stimulated the awareness to start an implementation on lower toxic, safer and eco-friendly pesticides. Besides, this problem which is associated with the continuous use of synthetic pesticides have led to the utilization of plant-based pesticides. This is due to a natural insecticidal component in plants that offers high selective toxicity to insects with limited off-target consequences. In addition, there are many benefits provided by the botanical insecticides such as low cost, less toxic to living organisms, eco-friendly, more energy- efficient and high yields in production. The use of biopesticides also are in purpose to promote agricultural practices in society. The neem plant was reported as the top list of herbs that have a potential to act as a biopesticides, this is due to the existence of multiple active compounds which are known as limonoids. The major compound is the azadirachtin which plays a vital role as insecticides and it kills the targeted pests by disturbing their growth and reproduction system. Furthermore, this neem oil-based pesticides are able to replace the use of synthetic pesticides due to its capability to give comparable effects toward agricultural production. It also can be considered more sustainable than chemical-based pesticides in which the by-product from the extraction process can be recycled back or make it as a biofertilizer. The neem oil biopesticides also are less toxic towards living organisms and less pollution toward the environment. Thus, the neem extracted biopesticides are now commercialized and have been sold and used in various countries. The neem extraction as biopesticides also need further studies and development to improve its weakness for a better performance to continuously provide sustainable practices in agriculture sectors.

References

[1] Srivastava, S. & Bhargava, A. (2012). Introduction to biotechnology. Biotechnology: New Ideas, New Developments (A Textbook of Modern Technology). 1-28.

[2] Singh, Ram. (2014). Industrial Biotechnology: An Overview. (December). Retrieved from https://www.researchgate.net/publication/311576484_Industrial_Biotechnology_An_Overview.

[3] Soetaert W & Vandamme E. (2006). The impact of industrial biotechnology. Biotechnology Journal, 1, 756-769. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.459.3632&rep=rep1&type=pdf.

[4] The Fankaso Women Marketing Federation. n.d. A Guide to Organic Pesticide preparation from Neem seed extract. Brikamaba, Gambia: UNDP. Retrieved from https://sgp.undp.org/publications-188/720-organic-pesticide-preparation-guide/file.html.

[5] Francis, C.A. (2004). Organic Farming. Encyclopedia of Soils in the Environment, 4(1994),77-84. Retrieved from https://sci-hub.tw/10.1016/b0-12-348530-4/00285-x.

[6] Fuad et al. (2012). The impact of pesticides on paddy farmers and ecosystem. Advances in Natural and Applied Sciences, 6(1), 65-70. Retrieved from http://www.academia.edu/download/28687300/IMPACT_OF_PESTICIDE_ON_PADDY_FARMERS.pdf.

[7] Resosudarmo, BP. (2004). Impact of the integrated pest management program on the Indonesian economy. (January 2001). Retrieved from https://www.researchgate.net/publication/4904

236_Impact_of_the_Integrated_Pest_Management_Program_on_the_Indonesian_Economy.

[8] V. Kandpal. (2014). Biopesticides. International Journal of Environmental Research and Development, 4(2), 191-196. Retrieved from https://www.ripublication.com/ijerd_spl/ijerdv4n2spl_15.pdf.

[9] Campos et al. (2016). Neem oil and crop protection: From now to the future. Frontiers in Plant Science, 7(October 2016), 1-8. Retrieved from https://doi.org/10.3389/fpls.2016.01494.

[10] Bajwa, AA & A, Ahmad. (2012). Potential applications of Neem based products as biopesticides. 3(4), 116-120. Retrieved from https://www.researchgate.net/publication/263973149_Potential_applications_of_Neem_based_products_as_biopesticides.

[11] Sporleder, M., & Lacey, L. A. (2013). Biopesticides. Insect Pests of Potato, 463–497. doi:10.1016/b978-0-12-386895-4.00016-8

[12] Boeke, S. J., Boersma, M. G., Alink, G. M., van Loon, J. J.., van Huis, A., Dicke, M., & Rietjens, I. M. C.. (2004). Safety evaluation of neem (Azadirachta indica) derived pesticides. Journal of Ethnopharmacology, 94(1), 25–41. doi: 10.1016/j.jep.2004.05.011

[13] Roychoudhury, R. (2016). Neem Products. Ecofriendly Pest Management for Food Security, 545–562. doi:10.1016/b978-0-12-803265-7.00018-x

[14] Winrock International (1998). Use of Neem as a Biological Pest Control Agent. Retrieved from: https://www.winrock.org/factnet-a-lasting-impact/fact-sheets/use-of-neem-as-a-biological-pest-control-agent/.

[15] Agbo, B. & And A & Ajaba, Mathias. (2015). A REVIEW ON THE USE OF NEEM (Azadirachta indica) AS A BIOPESTICIDE. Journal of Biopesticide and Environment. 2. Retrieved from https://www.researchgate.net/publication/289672873_A_REVIEW_ON_THE_USE_OF_NEEM_Azadirachta_indica_AS_A_BIOPESTICIDE.

[16] Hossain, Md & Khalequzzaman, M. (2018). Repellent and oviposition deterrent activity of leaf extracts of Azadirachta indica A. Juss., Persicaria hydropiper (L.) Spach. and Vitex negundo Linn. against the melon fruit fly, Bactrocera cucurbitae (Coquillett) (Diptera: Tephritidae). Retrieved from https://www.researchgate.net/publication/325473515_A_REVIEW_ON_THE_USE_OF_NEEM_Azadirachta_indica_AS_A_BIOPESTICIDE.

[17] Kumari, Archana & Kaushik, Nutan. (2016). Oviposition Deterrents in Herbivorous Insects and their potential use in Integrated Pest Management. Indian journal of experimental biology, 54. 163-174. Retrieved from https://www.researchgate.net/publication/298715783_Oviposition_Deterrents_in_Herbivorous_Insects_and_their_potential_use_in_Integrated_Pest_Management.

[18] Purrington, C. B. (2003). SECONDARY PRODUCTS | Antifeedant Substances in Plants. Encyclopedia of Applied Plant Sciences, 1140–1145. doi:10.1016/b0-12-227050-9/00127-7

[19] Chaieb, Ikbal. (2010). Saponins as Insecticides: A Review. Tunisian Journal of Plant Protection. 39. Retrieved from https://pdfs.semanticscholar.org/7a0b/2544dd484b919fd6afc0e5735759698d51d9.pdf.

[20] Liu, L., Zhao, Y.-L., Cheng, G.-G., Chen, Y.-Y., Qin, X.-J., Song, C.-W., … Luo, X.-D. (2014). Limonoid and Steroidal Saponin from Azadirachta indica. Natural Products and Bioprospecting, 4(6), 335–340. doi:10.1007/s13659-014-0042-2

[21] Mancebo, F., Hilje, L., Mora, G. A., & Salazar, R. (2002). Biological activity of two neem (Azadirachta indica A. Juss., Meliaceae) products on Hypsipyla grandella (Lepidoptera: Pyralidae) larvae. Crop Protection, 21(2), 107–112. doi:10.1016/s0261-2194(01)00069-2

[22] Agbenin, N.O. & Paul S., Marley. (2006). In-vitro assay of some plant extracts against Fusarium oxysporum f. sp. Lycopersici causal agent of tomato wilt. Journal of Plant Protection Research (Poland) 46,117 – 121. Retrieved from http://www.plantprotection.pl/In-vitro-assay-of-some-plant-extracts-against-Fusarium-oxysporum-f-sp-Lycopersici,90820,0,2.html.

[23] Silva, M. A., Bezerra-Silva, G. C. D., Vendramim, J. D., & Mastrangelo, T. (2012). Inhibition of Oviposition by Neem Extract: A Behavioral Perspective for the Control of the Mediterranean Fruit Fly (Diptera: Tephritidae). Florida Entomologist, 95(2), 333–337. doi:10.1653/024.095.0214

[24] Prakash, Kumari & Shipra. (2019). Biopesticides: Introduction and its Prospects. International Journal of Current Microbiology and Applied Sciences, 8(2), 2960-2964. Retrieved from https://www.ijcmas.com/8-2-2019/Nishant%20Prakash,%20et%20al.pdf.

[25] Benelli et al. (2017). Neem (Azadirachta indica): towards the ideal insecticide. Natural Product Research, 31(4), 369-386. Retrieved from https://doi.org/10.1080/14786419.2016.1214834.

[26] Marcello Nicoletti, Oliviero Maccioni, Tiziana Coccioletti, Susanna Mariani and Fabio Vitali (2012). Neem Tree (Azadirachta indica A. Juss) as Source of Bioinsectides, Insecticides - Advances in Integrated Pest Management, Dr. Farzana Perveen (Ed.), ISBN: 978-953-307-780-2, InTech. Retrieved from http://www.intechopen.com/books/insecticides-advances-in-integrated-pest-management/neem-treeazadirachta-indica-a-juss-as-source-of-bioinsectides

[27] Oguh et al. (2019). Natural Pesticides (Biopesticides) and Uses in Pest Management-

A Critical Review. Asian Journal of Biotechnology and Genetic Engineering, 2(3), 1-18. Retrieved from https://www.researchgate.net/publication/338165822_Natural_Pesticides_Biopesticides_and_Uses_in_Pest_Management-A_Critical_Review.

[28] Brahmachari. (2004). Neem - An omnipotent plant: A retrospection. ChemBioChem, 5(4), 408-421. Retrieved from https://sci-hub.tw/10.1002/cbic.200300749.

[29] Raizada et al. (2001). Azadirachtin, a neem biopesticide: Subchronic toxicity assessment in rats. Food and Chemical Toxicology, 39(5), 477-483. Retrieved from https://sci-hub.tw/10.1016/S0278-6915(00)00153-8.

[30] Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. Crop Protection, 26(9). 1337–1348. doi: 10.1016/j.cropro.2007.03.022

[31] United States Environmental Protection Agency (2012). Label Amendment to Split into Residential and Commercial Labels (Mimicking EPA Reg No. 84185-4, the Parent Product of this 100% Repack). Retrieved from https://www3.epa.gov/pesticides/chem_search/ppls/084181-00002-20130304.pdf.

[32] Baboo, Prem. (2016). Advancement in Neem Oil Extraction Process. Retrieved from https://www.researchgate.net/publication/292392229_Advancement_in_Neem_oil_Extraction_Process

[33] Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ, Sehgal R and Kanwar JR. (2017) Progress on Azadirachta Indica Based Biopesticides in Replacing Synthetic Toxic Pesticides. Front. Plnt Sci. 8:610. Doi: 10.3389/fpls.2017.00610

[34] Koul, O., Singh, G., Singh, R., Singh, J., Daniewski, W., and Berlozecki, S. (2004). Bioefficacy and mode-of-action of some limonoids of salannin group from Azadirachta indica A. Juss and their role in a multicomponent system against lepidopteran larvae. J. Biosci. 29, 409–416. doi: 10.1007/BF02712112

[35] Pathak, D. & Yadav, Rashmi & Kumar, Mukesh. (2017). Microbial Pesticides: Development, Prospects and Popularization in India. 10.1007/978-981-10-6593-4_18.

[36] Okolo, Emmanuel & Oyewole, Charles. (2019). Insecticidal Effect of Neem (Azadirachta Indica) Extracts Obtained from Leaves and Seeds on Pests of Cowpea (Vigna Unguiculata). Sumerianz Journal of Agriculture and Veterinary, 2(4). 20-28. Retrieved from https://www.researchgate.net/profile/Charles_Oyewole/publication/334454760_Insecticidal_Effect_of_Neem_Azadirachta_Indica_Extracts_Obtained_From_Leaves_and_Seeds_on_Pests_of_Cowpea_Vigna_Unguiculata/links/5d2b8f06299bf1547cb7ce06/Insecticidal-Effect-of-Neem-Azadirachta-Indica-Extracts-Obtained-From-Leaves-and-Seeds-on-Pests-of-Cowpea-Vigna-Unguiculata.pdf.

[37] Naniwadekar, MY. (2014). Process Development of Pesticide Production from. Journal of Agriculture Innovations ans Research, 1(3). 1473-2319. Retrieved from https://www.researchgate.net/publication/263275349_Process_Development_of_Pesticide_Production_from_Azadirachta_Indica_A_Juss

[38] Mendez et al. (2011). Production cost analysis and use of pesticides in the transgenic and conventional corn crop [Zea mays (L.)] in the valley of San Juan, Tolima. GM crops, 2(3), 163-168. Retrieved from https://sci-hub.tw/10.4161/gmcr.2.3.17591