

## CHAPTER ONE

### INTRODUCTION

Yam (*Dioscorea* spp) belongs to the family *Dioscoreaceae* and is a perennial herbaceous vine cultivated mainly for the consumption of its starchy tubers (Okigbo, 2004). It is one of the most important staple foods in the world, especially in some parts of tropics and subtropics (Okigbo and Ogbonnaya, 2006). The most cultivated species in Nigeria are *Dioscorea rotundata* (white yam), *Dioscorea cayenensis* (yellow yam) and *Dioscorea alata* (water yam) (Amusa, 1999). These edible varieties of yam are important food crops and serve as an important carbohydrate staple for millions of people in Sub-Saharan Africa, the Carribeans, the Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania (Coursey, 1967). More than 90% of the yam consumed in the world is produced in West Africa. Yam production has increased steadily in the last decades from 18 million metric tonnes in 1990 to a recent estimate of over 39 million metric tonnes (FAO, 2001). Nigeria alone produces three quarter of the world total output of yams (Okigbo, 2004).

One of the most pressing problems facing the countries of the third world is food scarcity. It is reported that nearly 1 billion people are challenged by severe hunger in these nations of which 10% die from hunger-related complications. During the past four decades, food production has failed to keep pace with population growth in many African countries. One group of commodities that holds much potential for reversing this trend is the roots and tubers. But in Africa, data compiled by researchers show that more than 40% of these root and tuber crops are lost to rot annually (Anon. 2009). Hence a substantial part of this problem from hunger stems from inadequate agricultural storage and produce preservation from microbes-induced spoilages.

According to Arya (2010), of all losses caused by plant diseases, those that occur after harvest are the costliest.

Postharvest deterioration and rot caused by diverse microorganisms is regarded as the single most important factor militating against commercial yam production in Nigeria apart from lack of research for development and capacity building in yam-based researches (Onyeka *et al.*, 2011; Taiga, 2011). Most post harvest spoilage of yam tubers are caused by fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Rhizoctonia spp.*, *Penicillium oxalicum*, *Trichoderma viride* and *Rhizopus nodosus* , (Ogbonnaya, 2006; Okigbo and Odurukwe, 2009; Okigbo and; Sangoyomi *et al.*, 2009). These microorganisms cause huge losses in the quantity of produce; they also create a lot of other consequences on produce quality (Nwachukwu and Osuji, 2008). Some workers reported that spoilage organisms produce extra-cellular enzymes such as amylases, celluloses, polygalactunases and pectin-methyl esterases which degrade cell wall components of susceptible produce resulting in the emission of foul odour and water (Salami and Popoola, 2007; Amadioha, 2012; Oladoye *et al.*, 2013) Hence rots reduce the market value of affected produce, hamper the addition of value to them and prevent produce to complete their roles in the food chain. In order to keep these organisms under check, controls are employed which increase the cost of production.

There are myriads of reports by researchers on the antimicrobial potentials of plant materials but there is a dearth of information on their synergistic effects on rot producing organisms in root and tubers crop. Hence the need to carry out this research in order to develop the right combination of these plant materials for effective inhibition of microbial growth.

## 1.1. Justification of the Study

The use of chemicals as fungicides has proved effective in the control of these rot producing organisms but not without challenges. Chemicals are not just expensive, not readily available but they are also not eco-friendly. Hence the need for safer, more efficacious, economical, and non-polluting methods has stimulated the search for alternative methods to control plant diseases instead of chemical products (Stangarlin *et al.*, 2011). The use of plants or plant products as fungicides is of great importance and needs more attention. Various plant products like gum, oil and resins are used as fungicidal agents (Daoud *et al.*, 1990). The use of bio-fungicides may have minimum adverse effect on the physiological processes of plant and less environmental hazards. Biological fungicides, being plant products are easily convertible into a common organic material and may create fewer health problems compared to synthetic alternatives.

Post harvest rot of root and tubers has been a very serious problem to farmers as more than 40% of their harvest may be lost because of decay (Okigbo *et al.*, 2014). The situation has been made worse by the misuse of synthetic chemicals resulting in development of resistance in target organisms, these chemicals are also expensive and not ecofriendly. This has rendered the use of chemicals inadequate to control post harvest rot and has created the need for new antifungal agent that is more affordable and ecofriendly. It is therefore necessary to evaluate the role of plant products in combating rot causing microbes. Plant extracts have been used against fungal agents but their combined effects or synergy has not been studied.

The principal species of microorganisms associated with yam tuber rot in Nigeria include *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Sclerotia rolfsia* and *Fusarium solani*. These fungi were reported to be pathogenic to yam tubers. The use of chemicals has

helped in control of rots (Okigbo *et al.*, 2014) but due to problems such as chemical residues, biodegradation, pollution, high cost, non availability and hazard to the environment, farmers have totally failed to adopt them (Okigbo and Odurukwe, 2009) hence alternative control methods are employed.

There are reports on the use of plant extracts in controlling rot causing organisms but no study has been done on the synergistic effects of plant extracts in controlling yam rot. The inference of this study will offer valuable information on the need to use different mixtures of plant materials at certain concentrations to control microbial rot of yam.

## **1.2. Aim of the study**

The study was aimed at investigating the synergistic effects of some plant extracts on fungi rot of yam from Umudike in Abia State.

## **1.3. Objectives of the study.**

The specific objectives were to:

- i. Isolate and identify fungi responsible for post-harvest rot of yam tubers
- ii. Evaluate the effects of aqueous and ethanol extracts of *Cymbopogon citratus*, *Citrus sinensis*, *Occimum gratissimum*, *Azadirachta indica*, *Carica papaya* and *Vernonia amygdalina* extracts on post harvest spoilage of yam
- iii. Determine the synergistic effects of combining the plant extracts in preventing post-harvest rot of yam
- iv. Evaluate the phytochemical constituents of the plant extracts.

## CHAPTER TWO

### 2.0. LITERATURE REVIEW

#### 2.1. Fungi responsible for postharvest rot of yam tubers

The invasion of yam tubers by microbial pathogens is considered most critical factor in yam decay (Degras, 1993). Pathogens such as fungi, bacteria, and nematodes cause postharvest losses of yam tubers through rotting. A number of fungal organisms have been implicated in the rot of stored yam tubers and they include: *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Rhizoctonia* spp., *Rhizopus nodosus*, *Penicillium oxalicum* and *Trichoderma viride* (Aidoo, 2007; Okigbo and Ikediugwu, 2002). Rottenness negatively affects the quality attributes of yam, it can lead to loss in flavor and in some cases the tubers are rendered unwholesome for consumption thereby, causing huge loss in market value. Even though bacteria do cause yam to rot, they are not economically important as mould fungi (Jonathan *et al.*, 2011).

Ezeibekwe *et al.*, (2016) reported that most rots of yam tubers are caused by pathogenic fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Rhizoctonia* spp., *Penicillium oxalicum*, *Trichoderma viride* and *Rhizopus nodosus*. This agrees with an empirical study conducted by several researchers who implicated the above listed organisms as responsible for post-harvest spoilage of yam (Okigbo and Ikediugwu, 2000; Okigbo, 2004; Aidoo, 2007; Agu *et al.*, 2014). The inference of a study conducted by Ogaraku and Usman (2008) on the storage rot of yam in Keffi, Nasarawa State showed that the fungi isolated included *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Rhizoctonia* spp with the following percentage frequencies of occurrences respectively: 38.6%, 19.3%, 18.1%, 20.0%, 7.2% and 4.8%., they further reported that there was no significant difference ( $P>0.05$ ) in the

incidence of the different isolates in relation to location. Similarly, Markson *et al.* (2012) isolated seven organisms (*Aspergillus niger*, *Penicillium expansum*, *Penicillium sclerotigenum*, *Fusarium solani*, *Botryodiplodia theobromae*, *Fusarium moniliformes* and *Rhizopus stolonifer*) as major rot-causing organisms of yam in Calabar, Cross River State, Nigeria. Of these, *Rhizopus stolonifer* was the most frequently isolated and *B. theobromae* the most virulent recording a percentage rot severity of 57.5%. Okigbo *et al.* (2015) reported that the fungal isolates associated with deterioration of white yam tubers in Anambra State were *Sclerotium rolfsii* Sacc, *Botryodiplodia theobromae* Pat, *Fusarium oxysporium* Schlect, and *Aspergillus niger* Van Tieghem. These pathogens were frequently isolated from rotten white yam tubers and these organisms have been reported to cause rots of yams. The pathogenicity test revealed that *Sclerotium rolfsii*, *Botryodiplodia theobromae* and *Fusarium oxysporium* induced rot in white yam tuber with *Sclerotium rolfsii* being most virulent. This agrees with the reports of some researchers on yam tubers (Sangoyomi 2004; Okigbo *et al.*, 2009a). The pathogenicity test also revealed that *Aspergillus niger* was a secondary pathogen to the rotten white yam. The isolation of more than one pathogenic microorganism from a particular yam tuber sample shows the possibility of multiple infections whose joint effect may lead to severe rotting of tuber crops (Okigbo *et al.*, 2015). These pathogenic fungi gain entry into yam tubers through natural openings and wounds created during harvesting, transportation, handling and marketing of the yam tubers. However, Okigbo and Nmeka (2005) reported that the root and tuber crops at the time of harvest may already be infected by pathogens derived from disease foliage, roots or mother tubers.

## **2.2. Effects of plant extracts on fungi responsible for yam rot**

Several methods have been adopted for controlling losses due to post harvest disease of yam; these include the use of chemicals, biological methods of control, curing uses of natural

plant extracts, as reported by Amusa *et al.* (2003). Chemical methods of control have helped to reduce the rate of storage losses and also increase yield obtained, but the problem arising with the use of chemicals is that it is expensive, can cause environmental pollution and may also induce pathogen resistance. Biological control method has been preferred in some cases because it is selective with no side effect and cheap. Resistance to biological control is rare and biological control agents are self- propagating and self-perpetuating (Okigbo and Ikediugwu, 2000). Some plants are known to synthesize phytochemicals with antimicrobial activities and are used successfully in the control of diseases in humans and crops like yam, cowpea and rice (Bediako *et al.*, 2007). The advantages of these natural plant products includes their local availability, little or no toxicity to humans and simple preparation procedures. Pesticides of plant origin are specific biodegradable, cheap readily available and environmentally safe than synthetic chemicals. The report of an empirical study conducted by Okigbo and Nmeke (2005) showed that plant extracts such as *Xylopiya aethiopica* and *Zingiber officinale* contain antimicrobial agents that inhibited the proliferation of some spoilage fungi responsible for yam tuber rot in storage. Plant extracts have been used to control diseases in crops such as cowpea and banana (Okigbo and Emoghene, 2004).

The documentation of Okigbo *et al.* (2015) showed that ethanolic extracts were observed to be more effective than the aqueous extracts in the control of rot associated with root and tuber crops, this suggests that water was not able to dissolve all the principal compounds present in the plant materials as did the ethanolic extracts. The result Okigbo *et al.* (2009) showed that Ethanolic extracts were more effective in all the test fungi, this agrees with the report of Ekwenye and Elegalam (2005) on garlic who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds (phytochemicals) needed for antifungal activity. The difference in the fungal toxicity between

the extraction medium may also be as a result of the different susceptibility of each of the test pathogenic fungi to different concentrations of the extract. This also agrees with the report of some workers (Okigbo and Nmeko, 2005; Okigbo and Ogbonnaya, 2006; Okigbo *et al.*, 2009a Onifade, 2011; Okigbo and Odurukwe 2009).

### **2.3. Synergistic effects of plant extracts on microorganism inducing rot**

In recent years, pathogenic microorganisms have developed drug resistance, hence the need to combine these therapeutic substances for possible synergism. The result of a research conducted by Natchimuthu *et al.* (2012) revealed that the antimicrobial activity of aqueous and ethanolic leaf extracts of *Aegles marmelos*, *Aegles amara*, *Cassia auriculata* and *Cassia quadrangularis* showed lower inhibition zones when used alone than that of the extract combinations. The Zone of Inhibition reached 3.0 cm against certain pathogenic organisms by the combination of aqueous extracts of all the four plants used in the study, indicating the high potential of combined use of plant extracts against pathogenic microorganisms. There is a possibility of using plant extracts in combinations against pathogenic bacteria as has been observed from the results. Similarly, Betoni *et al.* (2006) in his study reported that the antimicrobial activity of plant extracts on pathogenic organisms was confirmed and synergism was possible with all the therapeutic substances tested. Tetracycline presented synergism with all the extracts; and the *C. citratus* extract, although with the lowest antimicrobial activity, presented a synergism profile similar to that of *Syzygium aromaticum*, whose extract showed a relatively high inhibitory capacity on *S. aureus* growth. The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and inhibitors of protein synthesis, however, the understanding of synergism mechanism is fundamental to development of pharmacological agents to treat diseases by *S. aureus* using medicinal plants



## **2.4. Origin and domestication of white yam**

Generally, yam is native to warmer regions of both southern and northern hemispheres (IITA, 2004). White yam (*Dioscorea rotundata*) originated in Africa and is the most widely grown and preferred yam species. Domestication has been a traditional farmers practice in West Africa (Scarcelli *et. al.*, 2005). Guinea yams (*Dioscorea cayenensis-rotundata* complex; *D. rotundata* Poir. and *D. cayenensis* Lam.) have been described as resulting from a process of domestication of wild yams of the section *Enantiophyllum* by African farmers (FAO, 1998; Mignouna and Dansi, 2003; Zaknayiba and Tanko, 2013). Guinea yams were domesticated about 7000 years ago and over the years farmers have selected genotypes that best suit their needs, and thus have generated a large number of traditional cultivars.

There is tremendous genetic variability in yam. Contrary to the situation in other crops where the deployment of improved varieties has led to loss of diversity and a narrowing of the genetic base, some kind of domestication of semi-wild yam species is still ongoing in West African countries, which continually augments the germplasm diversity (Mignouna and Dansi, 2003).

## **2.5. Yam storage Losses**

Yam tubers are perishable produce (Alhassan, 1994) and a conservative estimate indicates that about 40% of yams produced annually are lost to rot (Olurinola *et al.* 1992). This is to the detriment of producers, distributors, and consumers. Though agriculturists generally believed that tubers of yam store well, that is not the case since candid observations showed that postharvest losses are heavy (Asiedu and Alieu, 2010). The principal factors responsible for yam losses during storage are: the respiratory process of the living but dormant tubers which result in the conversion of the starch of the tubers into carbon and water, activities of pests such as insects, nematodes and rodents also contribute significantly to yam loss. The bulkiness of yam

tuber, its chemical composition and moderately high water content predispose it to degradation during long-term storage (Asiedu and Alieu, 2010). Losses occurring during storage of yam could be classified into quantitative and qualitative losses. Quantitative losses of yam include weight loss which is mostly due to moisture loss through transpiration and, thereby, leading to desiccation. Qualitative losses include dry matter losses (loss of nutrient content as a result of sprouting and respiration) and loss of nutritive quality. Available statistics indicate that weight loss after 3 months of storage ranges between 10-20% and 50% after 6 months of storage (Robertson and Lupien, 2008)

## **2.6. Causes of Post-Harvest Losses of Yam**

The storage of fresh yam tubers has been confronted with a major problem over the years. The factors that contribute to yam losses in storage are physiological and pathological (Imeh *et al.*, 2012; Ravi and Aked, 1996). Physiological activities in yam that lead to postharvest losses are transpiration and respiration which in turn contribute to weight loss, sprouting (turning of edible tuber carbohydrates to inedible sprout), and desiccation (Imeh *et al.*, 2012; Osunde, 2008). Pathogenic causes of postharvest yam deterioration include moulding (fungal growth) and bacterial infection. Physiological activities taking place in yam tubers in storage bring about some changes in their internal composition, thereby, resulting in loss of nutritional qualities (Osunde, 2008), and under normal storage condition can cause 10% losses within 3 months and up to 25% losses in 5 months (Robertson and Lupien (2008). Major causes of postharvest losses, according to (Ezeh, 1995), are weight loss, insect attack, microbial, and sprouting. Sprouts development, according to Osunde (2008), is a major cause of storage losses. Weight loss is greatly influenced by respiration and transpiration where the latter is accelerated by sprouting (IITA, 1995; Kader, 2005). Yams exported to EU market from Nigeria are usually characterized by rotting at the point of arrival with a wastage rate of 10 to 50% (Asante, 2002) and 25-50%

losses globally (Anon, 1993). During the early stages of storage, yam tubers do experience low rate of respiration which is followed by high respiration rate which coincides with sprouting (PH, 2004c). Furthermore, there is a close relationship between the amount of CO<sub>2</sub> produced and dry weight losses in yam tubers (Afoakwa and Sefa-Dedeh, 2001).

Fungi on their own cannot penetrate intact tubers, hence wounding agents such as pests through feeding activities and more importantly mechanical damage arising during harvesting, storage, transportation and handling are sufficient to provide entry for these fungal pathogens (Sangoyomi, 2004).

## **2.7. Agronomic characteristics of yam**

Yam is cultivated for its energy-rich tuber. Yam planting is adaptable to fairly fertile soils and can be intercropped with legumes such as cowpeas, soybeans and a variety of leafy vegetables. A well-drained, rich, loamy soil however is the most favorable. Yam requires a warm, humid climate; however, the crop possesses considerable drought resistance. It gives more calories per unit of land area than most crops and matures within seven months. On soils of average fertility, between 20 and 30 tones per hectare of tubers can be obtained, and up to 55 tones per hectare on fertile soils. It has quite demanding labour and maintenance requirements, such as tilling the soil around each plant to form mounds, to ensure a pulverized soil favorable for tuber development. Storage of tubers occurs after harvest in barns or heaps covered with grass. It is a climbing vine with large underground roots up to 10 feet (3.3 meters) long. These roots have many shapes and may be white, offwhite, or purple inside. Over 60 varieties of yams are grown and eaten in the Pacific. Yams do not grow in areas, where there is not enough soil. Yams must be kept free of weeds for the first 3 months. When the vines start to grow, they are usually trained to grow onto long poles. In smaller gardens, where space is sure, they may be trained onto fruit trees. Some varieties of yams twist around a pole to the right, others twist to the

left. After 9-12 months, the yams are ready for harvesting. They are harvested when the leaves are dry (FAO 2001).

## **2.8. Methods of Storing Yam Tubers**

Yam tubers are highly perishable once they are harvested and kept above the ground. Losses are principally due to rot which have been shown to deplete up to 40% in Nigeria (Anon. 2009). The implications are therefore serious particularly in relation to the availability of sufficient planting materials to sustain yearly cropping. Chukwu *et al.* (2008) noted that lack of good storage methods limits the versatility of uses for which root and tuber crops are suitable.

The three main conditions necessary for successful yam storage are aeration, reduction of temperature, and regular inspection of produce. Ventilation prevents moisture condensation on the tuber surface and assists in removing the heat of respiration. Low temperature is necessary to reduce losses from respiration, sprouting and rotting; however, cold storage must be maintained around 12-15°C below which physiological deterioration such as chilling injury occurs. Regular inspection of tubers is important to remove sprouts, rotten tubers, and to monitor the presence of rodents and other pests. In general, tubers should be protected from high temperatures and provided with good ventilation during storage. The storage environment must also inhibit the onset of sprouting (breakage of dormancy) which increases the rate of loss of dry matter and subsequent shrivel and rotting of tuber.

Farmers in Nigeria prefer to store harvested yam tubers for a while before selling to achieve good market price. However, farmers tend to encounter high storage losses and this in turn has caused an increase in the prices of yam tubers, thereby, compelling yam lovers to resort to the consumption of other root and tuber crops as a substitute (Tetteh and Saakwa, 1991). Harvested yam tubers are generally stored in traditional barns, cribs and heaps, and in ditches (Opara, 1999). Leaving the tubers in the ground until required is the simplest storage technique

practiced by rural small-scale farmers. When carried out on-farm, this type of storage prevents the use of the farmland for further cropping. Harvested yams can also be put in ashes and covered with soil, with or without grass mulch until required. The yam barn is the principal traditional yam storage structures in the major producing areas. Barns are usually located in shaded areas and constructed so as to facilitate adequate ventilation while protecting tubers from flooding and insect attack.

Cold storage, irradiation and use of sprout inhibitors are rarely used to preserve yam tubers in Nigeria (Gyamfi, 2002). Cold storage as another method of storing yam, usually results in chilling injury when stored at temperatures below 13°C (Osunde, 2008; Anon, 2004). Fresh yam tubers are treated in various ways before storage (Osunde, 2008). Examples of the treatments include use of postharvest fungicides, plant extracts and gamma irradiation while storage techniques include the use of yam barns, cold storage, and improved underground storage (Osunde, 2008).

## **2.9. Constraints to Yam Production**

Yam production is limited by a number of factors acting singly or in combination. Eke-Okoro (2005) pointed out that major constraints in the production of root and tuber crops includes lack of improved and disease/pest tolerant varieties, low tuber yield and poor storability.

## **2.10. Use of Chemicals in the control of plant diseases**

The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. This is true for agents used in the treatment of bacterial, fungal, parasitic, and viral infections and even for treatment of chronic diseases such as cancer and diabetes; it applies to ailments caused or suffered by any living organisms, including humans, animals, fish, plants and insects (Salim, *et al.*, 2008). No doubt, Chemicals have great potentials in controlling plant diseases in many

respects, and countless success stories have been recorded. Regrettably, the use of these chemicals has been accompanied by the rapid appearance of resistant strains. Resistance to synthetic compounds is now a global issue and has been called one of the world's most pressing public health problems. The increasing misuse of synthetic chemicals has led to an international public health nightmare, with increasing microbial resistance to many synthetic compounds that once readily controlled them (Salim, *et al.*, 2008). With each passing time microbes that defy not only single but also multiple synthetic chemicals have become increasingly common and extremely difficult to control.

## **2.11. Antifungal properties of plant extracts**

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grown wild in different parts of our country. In Nigeria, the use of different parts of several medicinal plants to cure various disorders has been in vogue from ancient times. Synthetic chemicals are generally used against pathogenic microbes (Salim, *et al.*, 2008). Conventional synthetic chemicals are strong substances, which if not used in precise way may cause harmful effects. In recent years, microbial resistance to synthetic chemicals has been commonly reported from all over the world. So the major thrust is to establish alternative antimicrobial agent in order to control pathogenic microorganisms.

## **2.12. *Azadirachta indica* (Neem)**

### **2.12.1. Botanical description of *Azadirachta indica***

*Azadirachta indica* is a member of the family Meliaceae. It is broad-leaved evergreen tree which can reach heights of 30 meters with a trunk girth of 2.5 meters and can live for over two centuries. Its deep root system is well adapted to retrieving water and nutrients from the soil

profile, but this deep root system is very sensitive to water logging. The neem tree thrives in hot, dry climates where shade temperatures often reach 5°C and annual rainfall ranges from 400 to 1.200 mm. The tree can withstand many environmental adversities including drought and infertile, stony, shallow, or acidic soils. The neem produces ellipsoidal drupes, which are about two centimeters in length, borne on axillary clusters. These fruits contain kernels that have high concentrations of secondary metabolites (Schmulterer. 1990).



Plate 1: Leaves of *Azadirachta indica*



### **2.12.2. Secondary metabolites of Neem**

Many biologically active compounds can be extracted from neem, including triterpenoids, phenolic compounds, carotenoids, steroids, and ketones. The tetranortriterpenoid azadirachtin has received the most attention as a pesticide because it is relatively abundant in neem kernels and has shown biological activity on a wide range of insects (Schmutterer, 1995). Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highest quantity and azadirachtin-E regarded as the most effective insect growth regulator. Many other compounds have been isolated and they showed antecedent activity as well as growth regulating activity on insects. Polar and non-polar extractions yield about 24 compounds other than azadirachtin that have at least some biological activity. This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the affected organisms. However, only four of the compounds in neem have been shown to be highly effective in their activity as pesticides: azadirachtin, salannin, meliantriol and nimbin (Jacobson, 1990). Effects on phytopathogens

Subsequent to the isolation of azadirachtin from neem seed kernels (Butlerworth and Morgan, 1968): extensive work has been done on the chemistry and pesticidal properties of compounds from the neem tree (Schmutterer, 1995). Information relating to the antifungal activities of compounds from neem is limited. Neem leaves have been shown to possess antifungal activity either by direct soil amendment or as their extracts, active against a number of phytopathogens (Locke, 1995).

### **2.12.3. Antifungal activity of *A. indica***

The application of higher concentration of chemicals in an attempt to control pathogenic fungi responsible for food spoilage increases the risk of high level toxic residues in the product, which is particularly serious because these food materials are consumed in a relatively short time

after harvest (Maleki *et al.*, 2011). This necessitated the need to use plant material with antifungal potential in controlling food spoilage. The result of research conducted by so many researchers on the antifungal potential of *A. indica* on various fungi responsible for food spoilage suggests that it has antifungal property. Mondali *et al.* (2009) reported that aqueous and ethanol extract of *A. indica* inhibited significantly ( $p < 0.01$ ) the growth of pathogenic fungi responsible for brown spot of rice but the alcoholic extracts of neem leaf was most effective in comparison to aqueous extract for retarding the growth of *Rhizopus* and *Aspergillus*. Amadioha (2003) also reported that the growth of both pathogenic fungi *Rhizopus* and *Aspergillus* was inhibited with crude aqueous and alcoholic extract of different aged leaves of *Azadirachta indica*. It was further stated that the inhibition of growth of both the fungus was more pronounced with ethanolic leaf extracts as compared to aqueous leaf extracts. Significant inhibition of growth of *Rhizopus* and *Aspergillus* observed in the artificial culture media containing older leaf extracts of *Azadirachta indica*. The differences in the toxicity of different extracts could be attributed to the presence of the active principles that are extracted by different solvents, which may be influenced by several factors such as age of plant, method of extraction and type of extracting solvent. The greater effectiveness of ethanolic as compared with water extract of the neem leaf may be due to differences in constituent extraction. It has been previously reported that the active ingredients of neem constitute mostly of triterpenoides, eg, Nimbin, Nimbidine, Azadirachtin etc (Amadioha, 2003).

## **2.13. *Vernonia amygdalina* (Asteraceae)**

### **2.13.1. *Vernonia amygdalina*: Description**

*Vernonia amygdalina*, a member of the Asteraceae family, is a small shrub that grows in the tropical Africa with petiolate leaf of about 6 mm diameter and elliptic shape. It is commonly

called “bitter leaf” because of its bitter taste. The bitterness can, however, be abated by boiling or by soaking the leaves in several changes of water. In Nigeria, it is known variously as Ewuro in Yoruba language, Onugbu in Igbo language, Oriwo in Bini language, Ityuna in Tiv, Chusar doki or fatefate in Hausa, while it is known as Etidot in Cross River State of Nigeria. The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins, and glycosides (Bonsi *et al.*,1995). The leaves are used as green leafy vegetable and may be consumed either as a vegetable (leaves are macerated in soups) or aqueous extracts used as tonics for the treatment of various illnesses (Igile *et al.*, 1995). In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections (Huffman, 2003). Many herbalists and native doctors in Africa recommend its aqueous extracts for their patients as treatment for varieties of ailments ranging from, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and *Diabetes mellitus* among others. Some of these and other uses have been verified experimentally and documented by various workers, thus providing scientific evidences to support many of these claimed health benefits (Cimanga *et al.*, 2004).



Plate 2: Leaves of *Vernonia amygdalina*

### **2.13.2. Botanical Classification of bitter leaf**

The plant is scientifically classified as belonging to the Kingdom Plantae. It is an angiosperm, of the order Asterales, of the family Asteraceae, genus *Vernonia*, and species *V. amygdalina*.

### **2.13.3. Compounds Isolated from *Vernonia amygdalina***

Several investigators have isolated and characterized a number of chemical compounds with potent biological activities from the leaves of *Vernonia amygdalina*. Some of the previously isolated constituents in *Vernonia amygdalina*. include: sesquiterpene lactones (Cimanga *et al.*, 2004), flavonoids like luteolin, luteolin 7-O-glucosides and luteolin 7-O-glucuronide, steroid glycosides and vernonioside A, B, A1, A2, A3, B2, B3 and A4. Edotides from the aqueous extract of the plant was characterized in (Cimanga *et al.*, 2004). Very recently, Owoeye *et al.* (2010) isolated and characterized a sesquiterpene lactone, epivernodalol, another elemanolide from the dichloromethane fraction of *Vernonia amygdalina*. Koul *et al.* (2003) isolated the compound epivernodalol from another species of the plant called *Vernonia lasiopus*.

### **2.13.4. Antifungal activity of *V. amygdalina***

The report of a study conducted by John *et al.* (2016) revealed that natural products from *V. amygdalina* are relatively broad spectrum, bio-efficacious, economical, and biodegradable and can be ideal for use as agrochemicals. They also stated that *V. amygdalina* inhibited the growth of three fungi responsible for the spoilage of tomato in Jos, Plateau State. The *Rhizopus stolonifer* was more susceptible to the treatment (plant extract) in all concentration level with mean effect of 4.75 mm after 24 hours and 7.25 mm after 48 hours, followed by *Geotrichum candidum* and then *Fusarium oxysporum* which was least susceptible. They all showed significant differences ( $P < 0.001$ ). Sensitivity test using different concentrations of extract of *Vernonia amygdalina* showed they significantly affected all the identified organisms with *R. stolonifer* showing the

highest inhibitory effect ( $P < 0.001$ ). Another study conducted by Bazie *et al.* (2014) showed revealed that *Vernonia amygdalina* showed good antifungal activity against *Colletotrichum musae* the Cause of Postharvest Banana Anthracnose.

## **2. 14. *Carica papaya*:**

### **2.14.1. Botanic Description of the family caricaceae:**

This group consists of about 25 species of semi-succulent trees native to tropical America. These trees have straight trunks and are topped with palmate leaves. The most popularly grown species is *C. papaya*, commonly known as the Papaya. The Papaya is a short-lived, evergreen plant that can grow up to 25 feet. Its hollow, fleshy, green or purplish trunk is marked with leaf scars. The Papaya rarely branches. The leaves grow in a spiralled cluster directly from the upper part of the stem on horizontal petioles (leaf stalks) 1 to 3 1/2 feet long. The leaves are deeply divided. The life of a leaf is 4 to 6 months. Male and female flowers are produced on different plants, though there are hermaphrodite forms in cultivation as well as forms that bear both male and female flowers on the same plant. The flowers are fleshy and waxy and have a light scent. The blossoms are followed by deliciously edible fruits, which, although technically a berry, resemble melons. They have yellowish, thin skin and yellowish, peach, or orange to orangish-red flesh with a central cavity filled with small, pea-like, black seeds. The fruit tastes like a combination of melons and peaches. Although these trees are grown mainly for their fruit, all parts of the tree contain latex from which papain, a digestive enzyme, is extracted. Papain breaks down protein in meat to make it tender; therefore Papaya can be used as a meat tenderizer (Orwa, 2009).



**Plate 3: Leaves of *Carica papaya***

### **2.14.2. Antifungal Activity of *Carica papaya***

The latex of papaya and Fluconazole has synergistic action on the inhibition of *Candida albicans* growth. This synergistic effect results in partial cell wall degradation (as indicated by transmission electron microscopy observations). Latex alone is statically effective on *C. albicans* when added to a culture during the exponential growth phase and approximately 60% was achieved. This fungistatic effect is the result of cell wall degradation due to a lack of polysaccharides constituents in the outermost layers of the fungal cell wall and release of cell debris into the culture medium (Krishna, 2008).

### **2.14.3. Nutritional Value and uses of *Carica papaya*:**

The papaya, papaw, or pawpaw is the fruit of the plant *Carica papaya*, the only species in the genus *Carica* of the plant family Caricaceae. It is native to the tropics of the Americas. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m tall, with spirally arranged leaves confined to the top of the trunk. The leaves are large, 50–70 cm in diameter, deeply palmately lobed, with seven lobes. The tree is usually unbranched, unless lopped. The flowers appear on the axils of the leaves, maturing into large fruit. The fruit is ripe when it feels soft and its skin has attained amber to orange hue. These nutritional values of papaya help to prevent the oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and G and an excellent source of vitamin C (ascorbic acid). The extracts of unripe *C. papaya* contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids. These nutritional values of papaya help to prevent the oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and G and an excellent source of vitamin C (ascorbic acid). The extracts of unripe *C. papaya* contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids (Aravind, 2013).



## 2.15. Citrus

### 2.15.1. Botany of *Citrus sinensis*

Sweet orange (*Citrus sinensis* L. Osbeck) (to distinguish it from closely related species like sour orange, *C. aurantium* *C. reticulata* and mandarin orange), is a small evergreen tree 7.5 m high and in some cases up to 15 m. It originated from southern China where it has been cultivated for many years, but is today grown commercially worldwide in tropical, semi-tropical and some warm temperate regions to become the most widely planted fruit tree in the world (Nicolosi *et al.*, 2000). Orange produces leathery and evergreen leaves of different shapes, ranging from elliptical to oblong to oval, 6.5-15 cm long and 2.5-9.5 cm wide, often bearing narrow wings on the petioles. It bears fragrant white flowers either singly or in whorls of 6, about 5 cm wide, with 5 petals and 20-25 yellow stamens. The small, white or purple scented hermaphroditic flowers produce nectar for pollination by insects. The fruit, which may be globose to oval is 6.5 to 9.5 cm wide, and ripens to orange or yellow. Anatomically, the fruit consists of two distinct regions: the pericarp also called the peel, skin or rind, and the endocarp, or pulp and juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell. The quantity of wax is dependent on the variety, climatic conditions and growth rate. A plethora of microflora consisting mainly of fungus and bacteria are present on the skin and more copious in damp climates. This justifies the need for appropriate washing of the fruit before eating or proceeding to extract juice and essential oils. The pericarp consists of the outer flavedo, or epicarp largely made of parenchymatous cells and cuticle. Embedded oil glands create terpenoid aromatic compounds such as valencene, limonene, and alpha/beta sinesenol (Sharon-Asa *et al.*, 2003). Beneath the epidermis is the flavedo, with its characteristic yellow, green or orange colour. The flavedo is very

fine and fragile containing oliferous vesicles on the inside which can be collected by scraping on the flavedo layer. The flavedo is a generally colorless, spongy inner layer of mesophyll that changes character and thickness throughout fruit development, properties that determine ease of peeling. The albedo, or mesocarp lying beneath the flavedo consists of tubular-like cells joined together to constitute the tissue mass compressed into the intercellular area. The albedo is rich in flavonoids, which if transferred to the juice imparts a bitter taste.

The flesh or pulp of the fruit is typically juicy and sweet, divided into 10 to 14 segments (although there are seedless varieties) and ranges in color from yellow to orange to red. The ripe fruit is classified as a hesperidium which is a type of berry with multiple seeds and is fleshy. Fleshy juice sacs accumulate sugars, organic acids and large amount of water, causing difficulties in the extraction of nucleic acids and proteins. The endocarp and the *carpels* in which the juice containing vesicles are found and which from a synthetic biology point of view should be considered as the liquid released by the cytoplasm and by the vacuoles in the vesicles' internal cells. A spongy tissue similar to that of the albedo constitutes the the greater part of the fruit.



**Plate 4: The peel of orange (*Citrus sinensis*)**

### 2.15.2. Oranges, human health and nutrition

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids, essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material (Di-Majo *et al.*, 2005). The nutritional importance of foods is due to the presence of these functional food ingredients and antioxidant nutraceuticals or phytochemicals. Phytochemicals are present in edible fruits and vegetables and when eaten potentially modulate human metabolism in a favourable manner, thereby prevent chronic and degenerative diseases (Tripoli *et al.*, 2007). Increase in fruits and vegetables consumption protects against degenerative pathologies such as cancer andtherosclerosis; as epidemiological surveys had shown an inverse relationship between dietary flavonoid intake from citrus and cardiovascular diseases (Di Majo *et al.*, 2005). Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits. Moreover, it is now appreciated that other biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibres are known to be helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity and coronary heart diseases (Di-Majo *et al.*, 2005).

**As antioxidant:** A high quality orange is one that is mature with good color intensity uniformly distributed over the surface. Such oranges must be firm with a fairly smooth texture and shape that is characteristic of the variety, free from decay, defects and other blemishes. The biological activity and the healthy effects of citrus flavonoids as antioxidants have been reported (Tripoli *et al.*, 2007). These group of pigments as found in plants and together with anthocyanin play a role

in flower and fruit colouration. Also, they are present in dietary fruits and vegetables and exercise their antioxidant activity in several ways, including the activities of metal chelation. Studies indicate that flavonoids are excellent radical-scavengers of the hydroxyl radical (Darmon *et al.*, 1990), due to their ability to inhibit the hydroxyl radical and donate hydrogen atom (Di-Majo *et al.*, 2005, Tripoli *et al.*, 2007). Oranges as excellent source of vitamin C, contain powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases. Consumption of foods rich in vitamin C improves body immunity against infectious agents and scavenging harmful, pro-inflammatory free radicals from the blood. Sweet orange contains a variety of phytochemicals like hesperetin and naringenin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator.

**Anti-inflammation:** Citrus flavonoids contain compounds with anti-inflammatory activity due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxygenase, and cyclooxygenase) that control the formation of the biological mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation. Flavonoid inhibition of the immune and inflammation responses can be associated with their inhibition of these enzymes (Tripoli *et al.*, 2007). Indeed, citrus flavonoids are able to inhibit the kinases and phosphodiesterases essential for cellular signal transduction and activation. They also affect the activation of a number of cells involved in the immune response, including T and B lymphocytes. Citrus flavonoids also prevent atherosclerosis, inhibiting the formation of atheroma. Tripoli *et al.* (2007) reported that hesperidin obtained from citrus cultures may have a potential therapeutical use as a mild anti-inflammatory agent, being also useful as a precursor of new flavonoids endowed with this activity (Da Silva *et al.*, 1994). Studies using mouse macrophage cells also show that hesperidin has an inhibitory effect on lipopolysaccharide (LPS)-

induced over expression of cyclooxygenase-2, inducible nitric oxide synthase (iNOS), overproduction of prostaglandin E2 and nitric oxide (NO).

**Anti-Cancer and anti-Arteriosclerosis:** Citrus flavonoids can prevent cancer through selective cytotoxicity, antiproliferative actions and apoptosis (Elangovan *et al.*, 1994). Flavonoids are antimutagenic, thus protects the DNA from damage by their ability to absorb ultraviolet light. They neutralize free radicals that promote mutations when they are generated near DNA. This has been shown in mice body irradiated with c-ray. Flavonoids can also protect the DNA by interacting directly with the tumoral agents, as in the induced chromosomal aberrations by bleomycin. The inhibitory effect of citrus flavonoids on tumoral development and cell proliferation by rat malignant cells, in cardiac and hepatic tissue of syngenetic rats have been reported (Bracke *et al.*, 1989). The ability to function as such by citrus flavonoids are based on cell mobility inhibition. Oranges are also rich in iron, chlorine, manganese, zinc, sodium, phosphorous, iodine, calcium, folic acid, potassium, pectin, beta-carotene and amino acids and fibre. A single orange is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory blood clot inhibiting and antioxidant properties. All these properties help to promote overall health.

### **2.16. *Cymbopogon citratus* Lin.**

*Cymbopogon citrates* staff is popularly known as citronella grass or lemongrass. This species comprises approximately 500 genus and 8,000 herb species (Barbosa *et al.*, 2008). Lemon grass is a tufted perennial grass growing to a height of 1 meter with numerous stiff leafy stems arising from short rhizomatous roots. It has an economic lifespan for about 5 years (Carianne, 2005). The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm. The leaf-sheath is tubular in shape and acts as a pseudostem. Leaves are

long, glaucous, green, linear tapering upwards and along the margins. This plant produces flowers at matured stages of growth. Conversely, flowering has never been observed under cultivation due to rapid harvesting time. The inflorescence is a long spike about 1 meter in length. Flowers borne on decomposed spathe; panicles 30 to over 60 cm long. The rhizome produces new suckers that extend vertically as tillers to form dense clumps (Tajidin *et al.*, 2012).



Plate 5: Leaves of *Cymbopogon citratus*



### **2.16.1. Ethnobotany of *Cymbopogon citratus***

*Cymbopogon citratus* is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine. People nowadays are more aware on health issue due to the emergence of new diseases. Treatment using plant-based medicine appears to be an alternative approach due to the adverse effects associated with the use of synthetic drugs (Mirghani *et al.*, 2012). Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders. Studies have shown that the lemon grass has antibacterial and antifungal properties. Mixed with pepper, it's a home therapy for menstrual troubles and nausea. The lemon grass is a good cleanser that helps to detoxify the liver, pancreas, kidney, bladder and the digestive tract. It cuts down uric acid, cholesterol, excess fats and other toxins in the body while stimulating digestion, blood circulation, and lactation; it also alleviates indigestion and gastroenteritis. It is said that lemon grass also helps improve the skin by reducing acne and pimples and acts as a muscle and tissue toner. Also, it can reduce blood pressure. A recent study by the Food and Nutrition Research Institute of the department of Science and technology showed that lemon grass can help prevent cancer (Ojo *et al.*, 2006).

### **2.16.2. Antifungal activity of *Cymbopogon citratus***

Studies on the use of plant extracts such as *C. citratus* to control crop spoilage and phytopathogenic fungi are well documented (Aladi *et al.*, 2005). The antifungal activity of lemon grass oil has been tested against some species of pathogenic fungi (Abe, 2003). Essential oils from *C. citratus* have been tested for *in vivo* and *in vitro* antifungal activity and they demonstrated to be potential antifungal agents. Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell

death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration. Because of high volatility and lipophilicity of the essential oils, they are readily attached to penetrate into the cell membrane to exert their biological effect (Inouye, 2003). The result of a research conducted by Gawai (2015) of *Fusarium* spp depicted that the essential oil from the leaves of *C.citratius* is rich in antifungal compounds which possess considerable antifungal properties and a number of biological and medicinal potentials. It is good alternative to the harmful chemical pesticides and can be effectively used to control growth of *Fusarium* spp. Kumar *et al.* (2009) also reported that *C. citratius* essential oils exhibited broad fungitoxic activity against *Aspergillus flavus*. The result of an empirical research conducted by Tzortzakis and Economakis (2007) revealed that *C. citratius* oil inhibits the fungal spore production of *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, and *Rhizopus stolonifer*. On the same hand, Gupta *et al.* (2011) reported that *C. citratius* oil is a good alternative to the harmful chemical pesticides and can be effectively used as an efficient fungicide against *Fusarium* spp.

### **2.16.3. Phytochemistry and Pharmacology of *Cymbopogon citratius***

The use of medicinal plants is part of a competitive market, which includes pharmaceuticals, food, cosmetics, and perfumery markets (Rocha *et al.*, 2011). The chemical composition of the essential oil of *Cymbopogon citratius* varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered. Lemon grass contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol. The essential oil consists of, mainly, citral a volatile oil with strong lemon fragrance. Citral is a mixture of two stereoisomeric monoterpene aldehydes; the trans-isomer geranial (40-62%) dominates over the cis isomer neral

(25-38%) and is used in manufacture of perfumes, colored soaps and synthesis of Vitamin A (Shah, *et al.*, 2011).

**Anti-microbial activity:** The ethanolic extracts of the leaves of Lemon grass showed potential antibacterial property against *Staphylococcus aureus*. Flavonoids and Tannins found in the extract are responsible for the activity (Danlami, 2011).

**Anti-fungal activity:** *Candida albicans* is an important pathogen of human infections; moreover, other species can be associated with some infections. The anti-fungal activity of lemongrass and citral against *Candida* species was studied and the study showed that lemongrass oil and citral have a potent *in vitro* activity against *Candida* spp. (Silva *et al.*, 2008).

**Anti-protozoan activity:** The family Trypanosomatidae harbours protozoans that are agents of important illnesses in humans, animals and in plants. This family also includes some lower trypanosomatids such as *Crithidia*, *Blastocrithidia*, and *Herpetomonas*, monoxenous protozoans usually found in insect hosts. The essential oil extracted from *Cymbopogon citrates* showed anti-protozoan activity against *Crithidia deanei* (Pedrose *et al.*, 2006).

## **2.17. *Ocimum gratissimum* Linn.**

*Ocimum gratissimum* is also known as African Basil. It belongs to the Kingdom: Plantae, Order: Lamiales, Family: Lamiaceae, Genus: *Ocimum*, and Species: *Ocimum gratissimum*. Its vernacular names include: Ncho-anwu, Ahuji (Igbo), Efinrin (Yoruba), Aramogbo (Edo) and Daidoya (Hausa) (Effrain *et al.*, 2000). It is naturally used in the treatment of different diseases which includes: upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin diseases, pneumonia, tooth and gum disorder, fever, and as mosquito repellants (Ilori *et al.*, 1996). *Ocimum gratissimum* is a leafy vegetable and a good source of dietary fibre, carotenoids, vitamin C, foliate, photochemicals and certain minerals, but have low concentrations of proteins,

digestible carbohydrates and lipids. It is easy to cultivate and provides an inexpensive means of combating vitamin and mineral deficiency in less developed regions of the world. *Ocimum gratissimum* is found throughout the tropics and sub-tropics, both wild and cultivated. Its greatest variability occurs in tropical Africa, where it probably has its origin in India (Osuji *et al.*, 1995). It has interesting medicinal properties (Gill, 1992). It has been described to have other species in the flora of tropical West Africa (Nwinyi *et al.*, 2009). These include: *O. viride* Linn, *O. suave* Linn, *O. bacilicum* Linn and *O. canum* Sims.



Plate 6: Leaves of *Occimum gratissimum*

### **2.17.1. Morphology of *Ocimum gratissimum***

*Ocimum gratissimum* is a shrub up to 1.9m in height with stems that are branched. The leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubescent and dotted on both the sides. The leaves show the presence of covering and glandular trichomes. Stomata are rare or absent on the upper surface while they are present on the lower surface. Ordinary trichomes are few, while the long ones up to 6-celled are present on the margins mostly; the short ones which are 2 celled, are mostly found on the lamina. Petioles are up to 6 cm long and racemes up to 18 cm long. The peduncles are densely pubescent. Calyx is upto 5mm long, campanulate and 5-7 mm long, greenishwhite to greenish-yellow in colour. Nutlets are mucilaginous when they are wet (Bhat, 2003).

### **2.17.2. Traditional Uses of *Ocimum gratissimum***

*Ocimum gratissimum* has been used extensively in the traditional system of medicine in many countries. In the North east of Brazil, it is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion (Rabelo *et al.*, 2003). In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea (Effraim *et al.*, 2003). In the Savannah areas decoctions of the leaves are used to treat mental illness (Akinmoldun *et al.*, 2007). *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh, *et al.*, 2005). Brazilian tropical forest inhabitants use a decoction of *O. gratissimum* roots as a sedative for children (Cristiana *et al.*, 2006). People of Kenyan and sub Saharan African communities' use this plant for various purposes like viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they are also

used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Matasyoh *et al.*, 2007). In India, the whole plant has been used for the treatment of sunstroke, headache, influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity (Tania, *et al.*, 2006; Prajapati *et al.*, 2003). The tribes of Nigeria use the leaf extract in treatment of diarrhoea, while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu and Salau, 2005).

### **2.17.3. Medicinal Uses of *Ocimum gratissimum***

The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity (Celso *et al.*, 2002). A study on goats found that the essential oil has anthelmintic activity (Pessoa *et al.*, 2002). A test on guinea pigs found evidence that the essential oil relaxes the muscles of the small intestinal disorders (Socorro *et al.*, 2002). The ocimum oil is active against several species of bacteria and fungi (*Trichophyton rubrum* and *Trichophyton mentagrophytes*) (Obloh *et al.*, 2009). A previous screening of crude extracts of plants used in traditional medicine showed that the essential of *Ocimum gratissimum* inhibited growth of *Herpetomonas samuelpessoai* (Holetz *et al.*, 2002). African basil is used for a variety of reasons. In culinary, it is used in salads soups, pastas, vinegars and jellies in many parts of the world. The anti-diarrhea agent and for the treatment of conjunctivitis by instilling directly into the eyes; the leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis. The dried leaves are shuffled to alleviate headaches and fever among other uses (Iwu, 1990). Although, convectional antibiotics have been very useful in orthodox medicine it has been argued by many that its concomitant use with herbal extracts is not desirable as one

normally antagonizes the activity of the other. Considering the fact that *Ocimum gratissimum* is used in most local dishes or foods to achieve a variety of purposes, there is need to ascertain if its characteristics, antagonizes or acts as a synergy when used together with conventional antibiotics. In addition, despite the fact that the various extracts of *Ocimum gratissimum* have been tested *in vitro* and shown to be active against some bacterial and fungal isolates (Nakamura *et al.*, 2004; Silva *et al.*, 2005), specific strain differences supposes that a lot more status of bacteria and fungi across other regions be tested to ascertain their *in vitro* activity against this spice.

#### **2.17.4. Antifungal Activity of *O. gratissimum*.**

*Occimum gratissimum* is reported to have wound healing properties. Study was carried out to investigate the effect of honey as well as those of surfactants on the antibacterial activity of the essential oil of *O. gratissimum*. The antibacterial activity of dispersions of ocimum oil (2%) in methanol, honey, a macrogol blend, nonionic and ionic emulsifiers were assessed by cup-plate method using type bacteria and wound isolates. Honey enhanced the antibacterial activity of ocimum oil to a greater extent than the macrogol blend. The activity of ocimum oil emulsion in cetrimide (cationic) was lower than obtained for cetrimide solution. Emulsion of the oil in sodium lauryl sulphate (anionic) exhibited a slightly higher activity than the solution of the surfactant alone. Although Tween 20 (nonionic) and aqueous methanol had no activity, the emulsion of the oil in Tween 20 showed lesser activity than the oil solution in methanol. Honey's inherent antibacterial activity, surfactant charge interaction and the effect of emulsification were adduced to the observed differences in antibacterial activity of the ocimum oil formulations. Findings indicated that honey was a suitable base for ocimum oil especially in the treatment of infected wounds (Orafidiya *et al.*, 2006). An investigation of antifungal activity of the essential oil obtained by steam-distillation (1.1% w/w) of the aerial parts of *O. gratissimum* and of an



ethanolic extract from the steam-distillation residue was carried out using the agar diffusion method. The results revealed that the essential oil inhibited the growth of all fungi tested, including the phytopathogens, *Botryosphaeria rhodina*, *Rhizoctonia* sp. and two strains of *Alternaria* sp., while the extract from the residue was inactive. The antifungal activity of eugenol was evaluated against a species of *Alternaria* isolated from tomato and *Penicillium chrysogenum*. The minimal inhibitory concentrations of eugenol were 0.16 and 0.31 mg/disc for *Alternaria* sp. and *P.chrysogenum*, respectively (Terezinha *et al.*, 2006). Cryptococcal infection had an increased incidence in last few years' due to the explosion of acquired immunodeficiency syndrome. *O. gratissimum* has been reported earlier with *in vitro* activity against some bacteria and dermatophytes. *In vitro* activity of the ethanolic crude extract, ethyl acetate, hexane, chloroform fractions, essential oil, and eugenol of *O .gratissimum* was studied using an agar rdilution susceptibility method towards 25 isolates of *Cryptococcus mneoformans*. All the extracts of *O. gratissimum* studied showed activity *in vitro* towards *C. neoformans*. Based on the minimal inhibitory concentration values the most significant results were obtained with chloroform fraction and eugenol. It was observed that the chloroform fraction inhibited 23 isolates (92%) of *C. neoformans* at a concentration of 62.5 µg/ml and eugenol inhibited 4 isolates (16%) at a concentration of 0.9 µg/ml (Janine *et al.*, 2005). The antibacterial activity of different extracts from the leaves of *O. gratissimum* was tested against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Salmonella typhimurium*, pathogenic bacteria that cause diarrhoea. Extracts evaluated included cold water extract, hot water extract and steam distillation extract. Only the steam distillation extract had inhibitory effects on the selected bacteria and the minimum inhibitory concentration ranged from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium*, and 0.001% for *S. typhi* (Adebolu and Salau, 2005).

## **2.18. Phytochemical Constituents of Plant Materials**

Several authors have reported that plants contain a wide variety of bioactive constituents (Edeoga *et al.*, 2005). These bioactive constituents of plants have been demonstrated to possess antimicrobial properties. Banso and Adeyemo (2007) reported the antimicrobial activity of alkaloids, glycosides, flavonoids and tannins from plant stem bark. The research of plants' bioactive substances has contributed immensely for the betterment of mankind through the provision of value added economic returns, manufacturing of natural plant production industry, provision of better health care and increase in export earning in both rural and urban areas (Tona, 2002).

### **2.18.1. Phytochemical constituents of *Occimum gratissimum***

The result of Ameh (2010) confirmed that the leaf extract of *Occimum gratissimum* is rich in phytochemicals, the following phytochemicals were detected: glycosides, saponins, tannins, flavonoids, proteins, steroids and terpenoids. However, the extract of the plant material was devoid of alkaloids, resins, oils and acidic compounds. Terpenoids occurred in high concentrations. Glycosides and tannins were present in medium concentrations while saponins, flavonoids and steroids were found in low concentrations. On the same hand, the report of Udochukwu *et al.* (2015) showed that *V. amygdalina* and *O. gratissimum* contains the following phytochemicals: oxalate, phytate, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, anthraquinone, steroid and phenol. In terms of concentration in (mg/100g), *V. amygdalina* contained higher levels of bioactive compounds than *O. gratissimum* save for phytate and cyanogenic glycosides. The result of Ladipo *et al.* (2010) on the phytochemical analysis of *O. gratissimum* revealed the presence of alkaloids, tannins, saponin, steroids, cardiacglycoside, flavonoid, terpenoids and phenol. While the result of Nweze *et al.* (2004)

revealed the presence of alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpenes and flavonoids, this is similar to the results of Ladipo *et al.* (2010). Oladosu-Ajayi *et al.*, (2017) documented that *O. gratissimum* contains the same phytochemical compounds as the *V. amygdalina* but it was revealed from the results Erinle (2012) that more of these compounds can be found in the latter.

### **2.18.2. Phytochemical constituents of *Cymbopogon citratus***

The results of Umar *et al.* (2016) showed that phytochemicals abound in the leaf of *C. citratus*. The following phytochemicals were detected: flavonoids, carbohydrates, steroids, tannins, alkaloids, steroids, and phytosteroids) tested were all detected, except glycosides and phenols that were absent in the acetone and chloroform leaf extracts. This agrees with the findings of Sofowora (1984) and AOAC (1990), who reported that many results of phytochemical composition of the ethanol leaf extract of *Cymbopogon citratus* shows that it contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols, and flavonoids. Each of these phytochemicals is known for various protective and therapeutic effects. For instance, phenol is known to be an erythrocyte membrane modifier. Conversely the documentation of Mohammed and Bassem (2014) on the phytochemical constituent of *C. citratus* revealed that the constituents of lemon grass extracts mostly belong to monoterpene, sesquiterpene, and phenolic acids. This phytochemical composition analysis was found to be in accord with previously reported studies (Barbosa *et al.*, 2008; Negrelle and Gomes, 2007)

### **2.18.3. Phytochemical constituents of *Carica papaya***

Nath and Dutta (2016) documented that the phytochemical analysis of the leaves *Carica papaya* showed that the leaves contained tannin and saponins. The result showed that the levels of tannin and saponin in the plant extracts tested were 2.65% and 3.57mg/ml respectively. Tannins bind to produce rich protein and interfere with protein synthesis. They are known to

exert anti-microbial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). They are also observed to have remarkably activity in cancer prevention (Li *et al.*, 2003) showed tannins to be useful in treatment of inflamed or ulcerated tissues. The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic (Okwu and Okwu, 2004), since it contains tannin and saponin, (Gangwar *et al.*, 2015). Workers have found antimethanogenic activity in the papaya leaves. Tannin and saponin are responsible for reducing methanogenesis in rumen liquor. This is in contrast with the result of Ayoola and Adeyeye (2010) who reported that the phytochemical analysis of the leaves of *C. papaya* showed that the leaves contained saponins, cardiac glycosides, and alkaloids. Tannin was absent in the leaves. According to Ikeyi *et al.* (2013) *Carica papaya* leaves contain Alkaloid, Saponin, Tannin, Glycoside and Flavonoids and this is in agreement with the work of (Willson *et al.*, 2007). Also, from the result of Ikeyi *et al.* (2013), it was revealed that phytochemicals can be extracted more when the leaves are dried and pulverized to powdered form.

#### **2.18.4. Phytochemical constituents of *Citrus sinensis* (Orange peel)**

Osarumwense *et al.* (2013) reported that the phytochemical analysis of *C. sinensis* peel showed the presence of reducing sugar, saponins, cardiac glycosides, tannins and flavonoids. The presence of these constitute gives an indication of the medicinal value of the *Citrus sinensis* peels, for example, flavonoids have been found to have antioxidant properties, antibacterial and antimicrobial properties (Qian and Nihorimbere, 2004).

#### **2.18.5. Phytochemical constituents of *Vernonia amygdalina* (Bitter leaf)**

The reports of some researchers have shown that *V. amygdalina* contains varying quantities of the different phytochemicals. Offor (2014) reported that *V. amygdalina* contains high levels of flavonoids, saponins and  $\beta$ -Caroteinoids. On the same hand, the research of

Orjiakor *et al.* (2016) revealed that *Vernonia amygdalina* leaves contain anthracene, glycosides, steroids, flavonoids, proteins, carbohydrates, reducing sugars, saponins and tannins were present. While the documentations of Udochukwu *et al.* (2015) revealed that *V. amygdalina* contains bioactive compounds which include: oxalate, phylate, tannins, saponins, flavonoid, cyanogenic glycosides, alkaloids, anthraquinone, steroid and phenol, hence it can serve as good sources of useful elements and bioactive compounds. An empirical research conducted by Usunobun and Okolie (2016) on the biological active ingredient of *V. amygdalina* showed presence of flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids, reducing sugars and cardiac glycosides and absence of anthraquinones, the result also indicated that *Vernonia amygdalina* leaves, besides serving as good source of pharmacologically active phytochemicals may also be useful as supplements in human and animal nutrition. Other researchers proved that the leaf of *V. amygdalina* is rich in saponins, sesquiterpenes and flavonoids (Igile, 1994; Heftman, 1997, Sofowora, 2004).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1. Sources of Materials

The yam tubers with signs of rot and healthy yam (*Dioscorea rotundata* Poir) tubers were obtained Umuariaga market in Umudike Abia State Nigeria. The neem leaves (*Azadirachta indica* L.), bitter leaves (*Vernonia amygdalina* L.) and scent leaves (*Ocimum gratissimum* L.) were collected from a home garden in Abia state, while Paw-paw (*Carica papaya*), Lemon grass (*Cymbopogon citratus* L.) and orange bark (*Citrus sinensis* L.) were obtained from the staff quarters of The National Root Crops Research Institute, Umudike. The plants were identified by Prof. R. N. Okigbo and authenticated by Mr Tochukwu Egboka of the Department of Botany, Nnamdi Azikiwe University, Awka.

#### 3.2. Isolation of Fungal Pathogens

The method of Okigbo *et al.* (2015) was used for fungal isolation. The yam tubers with rot symptoms were washed with water and surface sterilized with 70% ethanol solution for 1 minute and rinsed with sterile distilled water. Then a sterile kitchen knife was used to cut open the yam tubers to reveal the rotten and healthy parts. Carefully, portions (about 3 mm in diameter) were taken at the boundary area of the healthy and infested part of the incised yam tuber and inoculated on the solidified Potato Dextrose Agar (PDA) medium in culture plates. The plates were sealed and incubated in an incubator at 25°C for 3-5 days. The plates were examined daily for the presence of fungal growth. Pure cultures were obtained by sub-culturing on a freshly prepared PDA for 3-5 days. The pure cultures were identified using a microscope and identification guide (atlas) to confirm the isolated organisms.

### 3.3. Pathogenicity Test and Identification of Isolates

The needle injection inoculation method of Suchitra and Shavannor (2014) was used to test the pathogenicity of the isolated organisms. The fungal isolates obtained from the rotten yam tubers were tested for their ability to cause the same rot condition in healthy yam tubers. The healthy yam tubers were washed with sterile water and the surface sterilized with 70% ethanol solution. With the aid of a sterile syringe, the pure culture of isolates was inoculated into a healthy yam tuber at different points. The inoculated tuber was kept in a micro humidity chamber at room temperature and examined daily for rot development for 7 days. On establishment of rot condition, re-isolation was carried out to obtain pure cultures of the inoculated isolate which was compared with original isolates before being characterized and identified as the casual organism.

The pathogenic organisms were subjected to microscopic examination during which their structural features were observed under the hand lens before being mounted on a slide mount and stained with cotton blue lacto-phenol and viewed under the microscope. The characteristic features observed were compared with those contained in Barnett and Hunter (1987) and identified accordingly. A pathogenicity test of each isolate was replicated three times. On appearance of symptoms, the area of infection was measured in millimeters using a metre rule and the mean percentage infection (disease severity) was calculated using the formula cited in Umana *et al.* (2015)

thus:

$$\text{Disease severity (Area)} = \frac{\text{Area of plant tissue affected}}{\text{Total area}} \times \frac{100}{1}$$

### **3.4. Preparation of Plant Leaf Extracts**

Fresh leaves of neem (*Azadirachta indica*), bitter leaf (*Vernonia amygdalina*), paw paw (*Carica papaya*), lemon grass (*Cymbopogon citratus*), scent leave (*Ocimum gratissimum*) and bark of orange (*Citrus sinensis*) respectively were thoroughly washed in running tap water and rinsed with sterile distilled water, air dried in the laboratory and milled with a milling machine (CNC Germany) to obtain a powdery form. Seven extract combinations of the milled part of the plant namely *A. indica* leaves and *V. amygdalina* leaves, *C. papaya* and *C. citratus*, *C. sinensis* leaves and *O. gratissimum* leaves, *A. indica* and *C. citratus*, *O. gratissimum* leaves and *V. amygdalina* leaves, *V. amygdalina* and *C. papaya* and *C. sinensis* and *A. indica* leaves were used for the study. Serial dilution method according to Doughari *et al.* (2009), was adopted by infusing 25g, 50g, 75g and 100g of each of the botanical mixtures in 100mls of sterile distilled water in 500 ml conical flask, they were thoroughly mixed together using sterile glass rod and left for 24 hours before being filtered into a fresh 500ml flask using the four fold cheese cloth as described by Wokocha and Okereke (2005). These preparations: 25g of solute/100ml of solvent, 50g of solute/100ml of solvent, 75g of solute/100ml of solvent and 100g of solute/100ml of solvent represent 25%, 50%, 75% and 100% of aqueous-extract concentrations respectively of all the extracts. The same procedure was also done for ethanol extract of all the plant materials.

### **3.5. *In vitro* Screening of plant extracts against radial fungal growth**

Effect of plant extract on mycelia growth of the six test fungi was studied using the food poisoning techniques (Sangoyomi, 2004). One milliliter of each plant extract concentrations (25%, 50%, 75% and 100%) was dispensed per Petri dish and 9 ml of molten PDA was added to each of the Petri dishes containing extract and carefully spread evenly over the plate, this gave rise to PDA-extract mixture with corresponding 2.5%, 5.0%, 7.5% and 10% extract



concentration. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the centre with a 4 mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of each of the six test fungi.

After inoculation, all the plates were incubated at 25<sup>0</sup>C for 5days and examined at the 5<sup>th</sup> day for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Okigbo *et al.* (2009)

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_2} \times \frac{100}{1}$$

Where R<sub>1</sub> is the farthest radial distance of Pathogen in control plate while R<sub>2</sub> is the farthest radial distance of Pathogen in extract incorporated agar plates.

### **3.6. Quantitative phytochemical screening**

This was aimed at determining the presence and quantity of phytochemical of interest in the plant materials; quantitative tests were conducted using different standard methods to determine the presence of alkaloid, saponin, tannins, and oxalate.

#### **3.6.1. Determination of Saponin by gravimetric oven dry method**

The method of Nweze and Nwafor, (2014) was adopted. Ten grammes of the test plant samples were weighed in an extraction tube; 100 mls of 20% aqueous-ethanol was added to the sample and shaken for 2 hours, then filtered through a No 1 Whatman filter paper. Residues were extracted with another 100 mls 20% extracting solution. The extract was placed on a hot plate and the volume was reduced to 50 ml. Twenty milliliters of diethyl-ethanol was added to the

sample and shaken vigorously after which it was transferred to a separating funnel. The aqueous layer was recovered while the ether discarded; 4 g of NaCl was added to the aqueous solution and was shaken for about 30 minutes with 100 ml of ethanol and placed in the separating funnel. The saponin dish was placed in the oven to dry to a constant weight. The saponin was calculated as follows: % saponin= weight of extract x 100

### **3.6.2. Determination of Tannins by Folin –Denis Spectrop-photometric Method.**

The method of Oludoru (2012) was used. One gramme of the sample was weighed; it was dispersed in 10ml distilled water and agitated. This was left to stand for 30 mins at room temperature, being shaken every 5 minutes; it was centrifuged to obtain the extract. 2.5 ml of the supernatant (extract) was dispersed into a 50ml volumetric flask. Similarly 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. 1.0ml folin –Denis reagent was measured into each flask, followed by 2.5 ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was diluted to 50ml mark in the flask using water and incubated for 90 mins at room temperature. The absorbance was measured in a Genway model 6000 electronic spectrophotometer. Reading was taken with the reagent blank at zero. The tannin content was calculated using the formular:

$$\% \text{ Tannins} = \frac{A_n \times A_s \times C \times 100}{W \times V_f \times V_A}$$

Where:

A<sub>n</sub> = absorbance of test sample, A<sub>s</sub> = absorbance of standard solution, C = Concentration of standard solution, W = Weight of sample used, V<sub>f</sub> = Total Volume of extract, V<sub>A</sub> = Volume of extract analyzed.

### 3.6.3. Alkaloid Determination by Gravimetric Method

The method of Okigbo *et al.* (2015) was applied. Five grammes of the sample was weighed and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixtures were shaken well and allowed to stand for 4 hours before filtering. The filtrate was evaporated to one quarter (1/4) of its original volume. Conc. NH<sub>4</sub>OH was added dropwise to precipitate the alkaloid. The Precipitate was filtered off with a weighed filter paper and washed with 1% NH<sub>4</sub>OH Solution the precipitate was dried in filter paper in the oven at 60<sup>0</sup>C for 30 mins and reweighed, then % Alkaloid was calculated as follows:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

W

Where: W = weight of sample, W<sub>1</sub>= weight of empty filter paper , W<sub>2</sub> = weight of paper plus precipitate

### 3.6.4. Total Flavonoids Content

The total flavonoids content was estimated using the procedure described by Zhishen *et al.* (1999). One milliliter (1 ml) of plant extracts were diluted with 200 µl of distilled water separately followed by the addition of 150 µl of sodium nitrite (5%) solution. This mixture was incubated for 5 minutes and then 150 µl of aluminium chloride (10%) solution was added and allowed to stand for 6 minutes. Then 2 ml sodium hydroxide (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left for 15 minutes at room temperature. The absorbance was measured at 510 nm. Apperance of pink colour showed the presence of flavonoid contents. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using standard curve.

### 3.6.5. Total Phenolic content

The total phenolics content of the different plant mixtures was estimated using Folin-ciocalteau reagent by the method of Sidduraju and Becker (2003). About 20 µg of leaf extracts were taken separately and it was made up to 1 ml with distilled water. Then 500 ul of diluted Folin-phenol reagent (1:1 ratio with water) and 2.5 ml of sodium carbonate Na<sub>2</sub>CO<sub>3</sub> (20%) were added. The mixture was shaken well and incubated in dark condition for 40 minutes for the development of colour. After incubation, the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 ug/ml. The total phenolics content in the plant-extracts were expressed as mg of gallic acid equivalent (mg/GAE/g extract) by using the standard curve.

### 3.6.6. Phytate Content Determination

Method of Njoku *et al.* (2016) was adopted. One gram (1g) of the test sample was weighed out into a 500 ml flat bottom flask. The flask with the sample was placed in a shaker and extraction was done with 100 ml of 24% Hydrochloric acid (HCl) for one hour at room temperature. The solution was decanted and filtered. Five milliliters (5 ml) of the filtrate was pipetted out and diluted to 25 ml with distilled water. Fifteen milliliters (15 ml) of sodium chloride was added to 10 ml of the diluted sample. The absorbance reading of the solution was taken at 520 nm.

$$\text{Concentration} = \frac{\text{Absorbance of sample}}{\text{Gradient Factor}} \times \text{DF}$$

Where DF= Dilution Factor, Gradient Factor = Slope of the standard curve

### 3.6.7. Oxalate Content Determination

Oxalate quantitative determination was carried out using the method reported by Ejikeme *et al.* (2014). Two grammes (2 g) of the sample was weighed into a 300 ml flask. Twenty milliliters (20 ml) of 30% hydrochloric acid (HCl) was added to the sample and allowed to stand for twenty min. Forty grams of ammonium sulphate was also added and allowed to stand for another thirty minutes. The solution was filtered (with Whatman's No 1 filter paper) into a 250 ml volumetric flask and made up to the mark with 30% HCl. Ten milliliters (10 ml) of the filtrate was transferred into 100 ml centrifuge tube. Thirty milliliters (30ml) of diethyl ether was added to it. The pH was adjusted to pH 7 with ammonium hydroxide. The solution was centrifuged at 10,000 rotations for fifteen minutes. It was decanted into a 250 ml conical flask and then titrated with 0.1M potassium tetraoxomanganate (IV) (KMnO<sub>4</sub>). The volume used (titre) was noted.

The percentage oxalate was calculated thus:

$$\% \text{ oxalate} = \frac{\text{titre mol KMnO}_4 \times DF \{12.5\} \times 100}{\text{Weight of sample}}$$

Where DF is the dilution factor

### 3.7. Analysis of Data

Data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan at 0.05 probability level to indicate the level of significance between values.

## CHAPTER FOUR

### RESULTS

#### 4.1. Fungal organisms isolated and identified from yam rot

The fungi isolated from the yam tubers with symptom of rot were identified based on their morphological and microscopic features (Table 1).

**Table 1: Morphological and microscopic features of fungal isolates from yam rot**

S/N	Species of fungi	Morphological features		Microscopic features
		Texture of growth	Colour on surface	Colour on reverse
1.	<i>Aspergillus niger</i>	Powdery, dense, felt of conidiospores	White to yellow which quickly turns to black	Turns from white to pale The hyphae are septate and clear with long conidiophore
2.	<i>Aspergillus flavus</i>	Rough texture	Greenish-yellow colour and looks wooly	Cream to yellow Has septate hyphae with long conidiophore.
3.	<i>Fusarium solani</i>	Smooth texture	Off-white cottony colonies	Pale yellow The hyphae are clear and non pigmented and are septate. The conidiophores are short
4.	<i>Penicillium spp</i>	Powdery with smooth walled ascospores	Slightly gray mixed with orange colour and sometimes with a white periphery outer edge	Pale yellow to light yellow Septate hyaline hyphae and smooth walled conidiophores
5.	<i>Rhizopus stolonifer</i>	Fluffy cotton appearance	Whitish	Turns Brown when aged The sporangiophore are located upon the collumella at the apical end of the sporangiophore
6.	<i>Botryodiplodia theobromae</i>	Smooth walled	Colour appear fluffy with aerial mycelium which becomes pale or grey as the culture ages	Gray Uniseptate, thick walled conidiomata covered with smooth hyphae

#### **4.2. Occurrence of fungi pathogens isolated from spoilt samples of yam**

The fungi pathogens that were consistently isolated from the rot infested tissues of the yam include *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Fusarium solani*, *Aspergillus niger*, *Penicillium* Spp and *Aspergillus flavus* (Table 1). The frequency of occurrence varied with different fungi associated with the spoilt yam. *Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Fusarium solani* occurred most frequently with 30.3, 26.0 and 16.4 percentages respectively while others had lower frequencies of occurrence from 5 to 12 percentages (Table 2).

Table 2: Frequency of occurrence (%) of isolated fungi on spoilt yam samples

<b>S/N</b>	<b>Fungal Isolate</b>	<b>Frequency (%)</b>
1	<i>Botryodiplodia theobromae</i>	30.3
2	<i>Rhizopus stolonifer</i>	26
3	<i>Fusarium solani</i>	16.3
4	<i>Aspergillus niger</i>	12
5	<i>Penicillium spp</i>	10.4
6	<i>Aspergillus flavus</i>	05



### 4.3. Pathogenicity Test

Five out of six fungal isolates namely *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Fusarium solani*, *Aspergillus niger* and *Aspergillus flavus* were pathogenic and caused rot in healthy yam tubers after seven days of inoculation. The most virulent was *Botryodiplodia theobromae* with rot incidence of 80 mm followed by *Rhizopus stolonifer* (75 mm). *Penicillium* spp was not pathogenic (Table 3).

Table 3: Pathogenicity Test of Test Isolates on healthy yam tubers

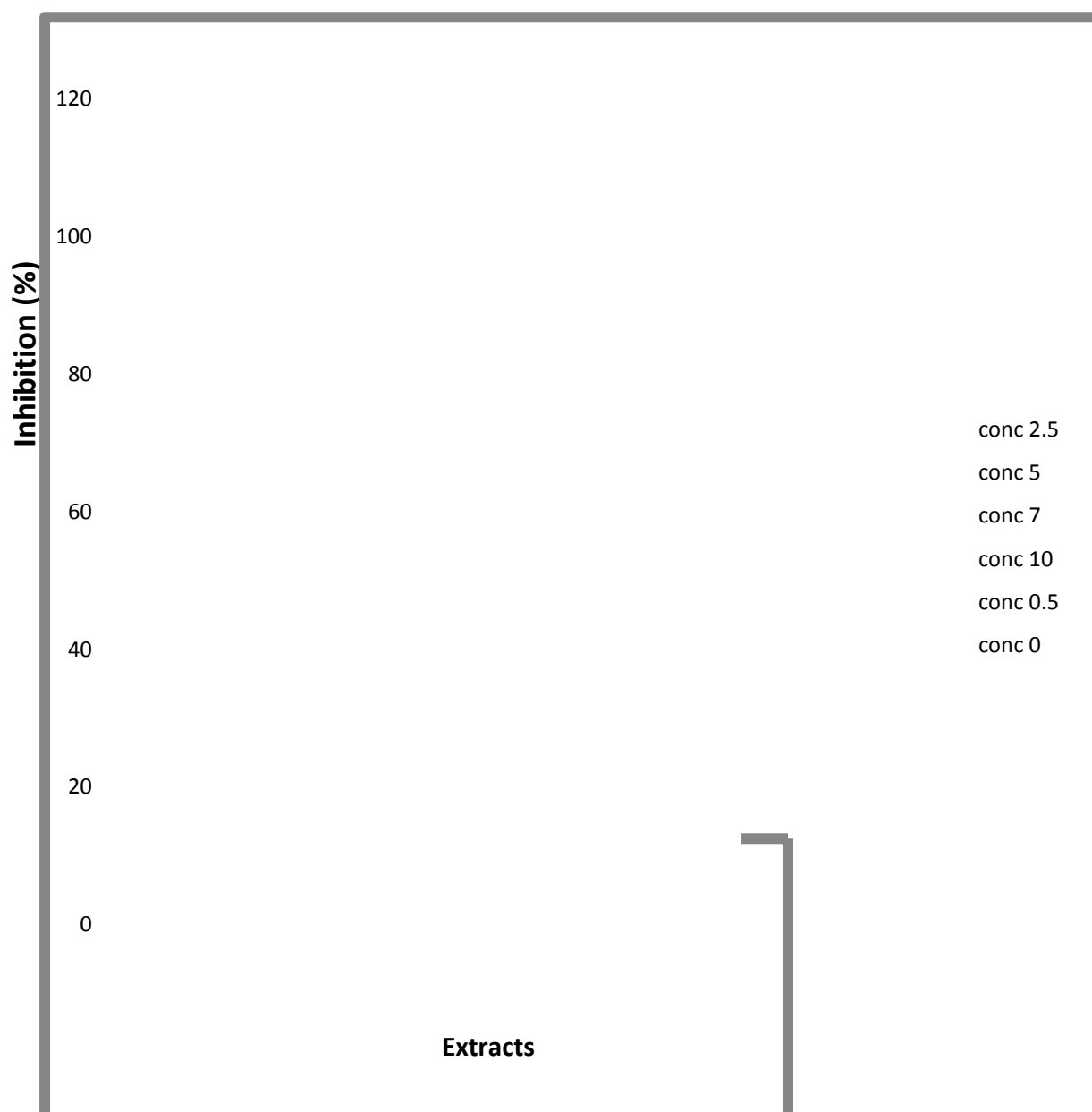
<b>S/N</b>	<b>Isolates/Inoculated Fungi</b>	<b>Rot (mm)</b>
1	<i>Botryodiplodia theobromae</i>	80
2	<i>Rhizopus stolonifer</i>	75
3	<i>Fusarium solani</i>	71
4	<i>Aspergillus niger</i>	51
5	<i>Penicillium spp</i>	0
6	<i>Aspergillus flavus</i>	39

#### 4.4. Effects of aqueous and ethanol extracts of plant materials on the inhibition of test

##### Organisms

The effects of plant extracts on the inhibition of all the fungi isolated varied with extraction medium and concentration. Most inhibitory effects were recorded at 7.5% and 10% extract concentrations than 2.5 and 5% concentrations while ethanol extract proved to be more potent than the aqueous extract.

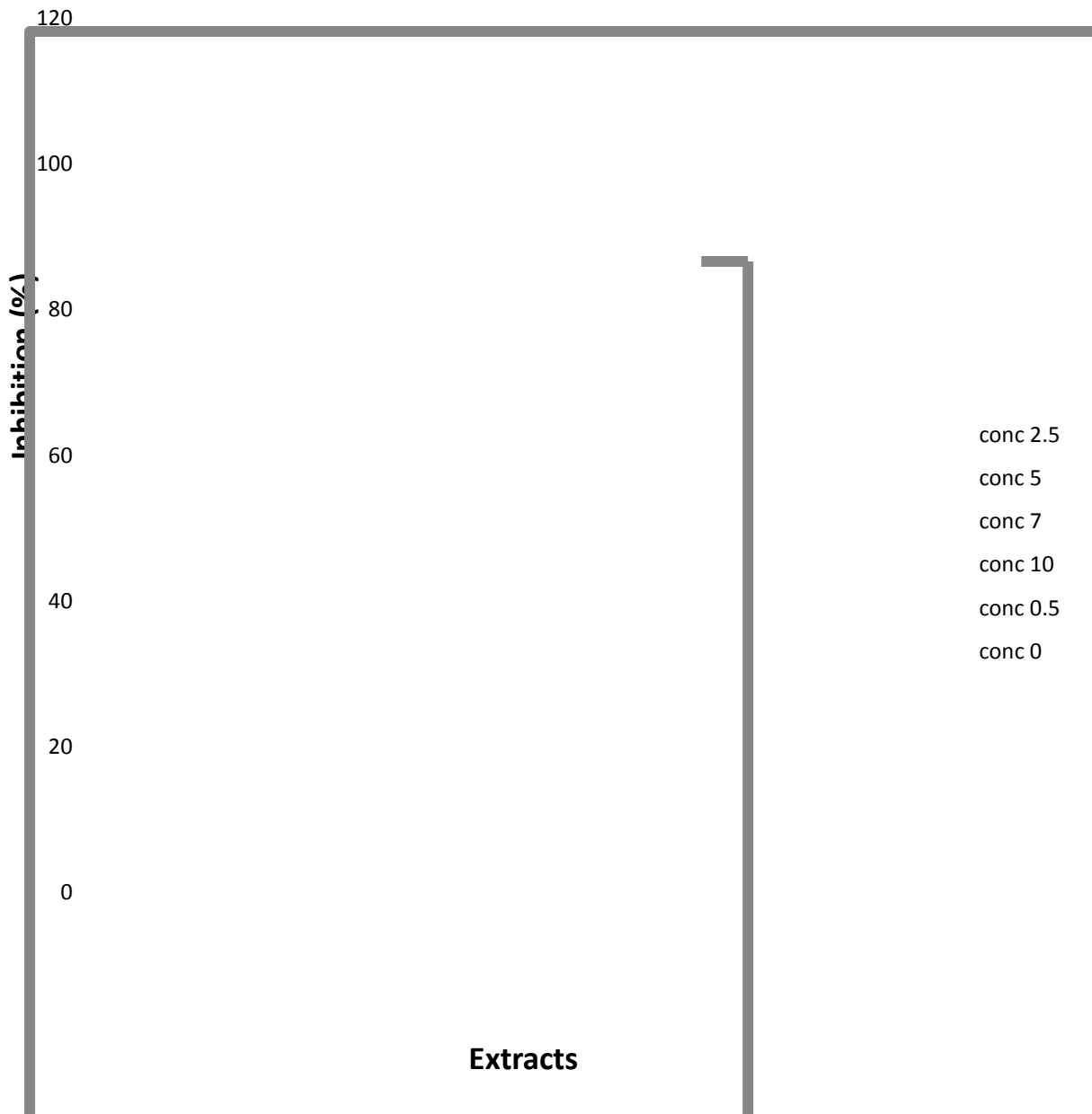
For *Rhizopus stolonifer* ethanol extract of *A. indica* at 7.5% and 10% concentration gave the highest inhibitory effect of 43% each, next to this were ethanol extracts of *O. gratissimum*, *C. citratus* and *V. amygdalina* at 7.5%, 10% with values of 42%, and 40% respectively. Inhibition recorded from all the aqueous extracts were relatively lower than values observed from ethanol extracts. The highest value observed from aqueous extract was from *A.indica* (37% inhibition) while the least value was recorded from aqueous extract of *O. gratissimum*. The commercial fungicide used gave the highest inhibitory value of (96%) (Figure 1).



**Figure 1: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Rhizopus stolonifer***

**Key:** **ET-Cymbo** means ethanol extract of *Cymbopogon citratus*; **ET-Occimum** means ethanol extract of *Occimum gratissimum*, **ET-Citrus** means ethanol extract of *Citrus sinensis*, **ET-Azadirachta** means ethanol extract of *Azadirachta indica*, **ET-Vernonia** means ethanol extract of *Vernonia amygdalina*, **ET-Carica** means ethanol extract of *Carica papaya*, **AQ-Cymbo** means aqueous extract of *Cymbopogon citratus*; **AQ-Occimum** means aqueous extract of *Occimum gratissimum*, **AQ-Citrus** means aqueous extract of *Citrus sinensis*, **AQ-Azadirachta** means aqueous extract of *Azadirachta indica*, **AQ-Vernonia** means aqueous extract of *Vernonia amygdalina*, **AQ-Carica** means aqueous extract of *Carica papaya*, **P-CTRL-MAN** is Mancozeb (Commercial fungicide),

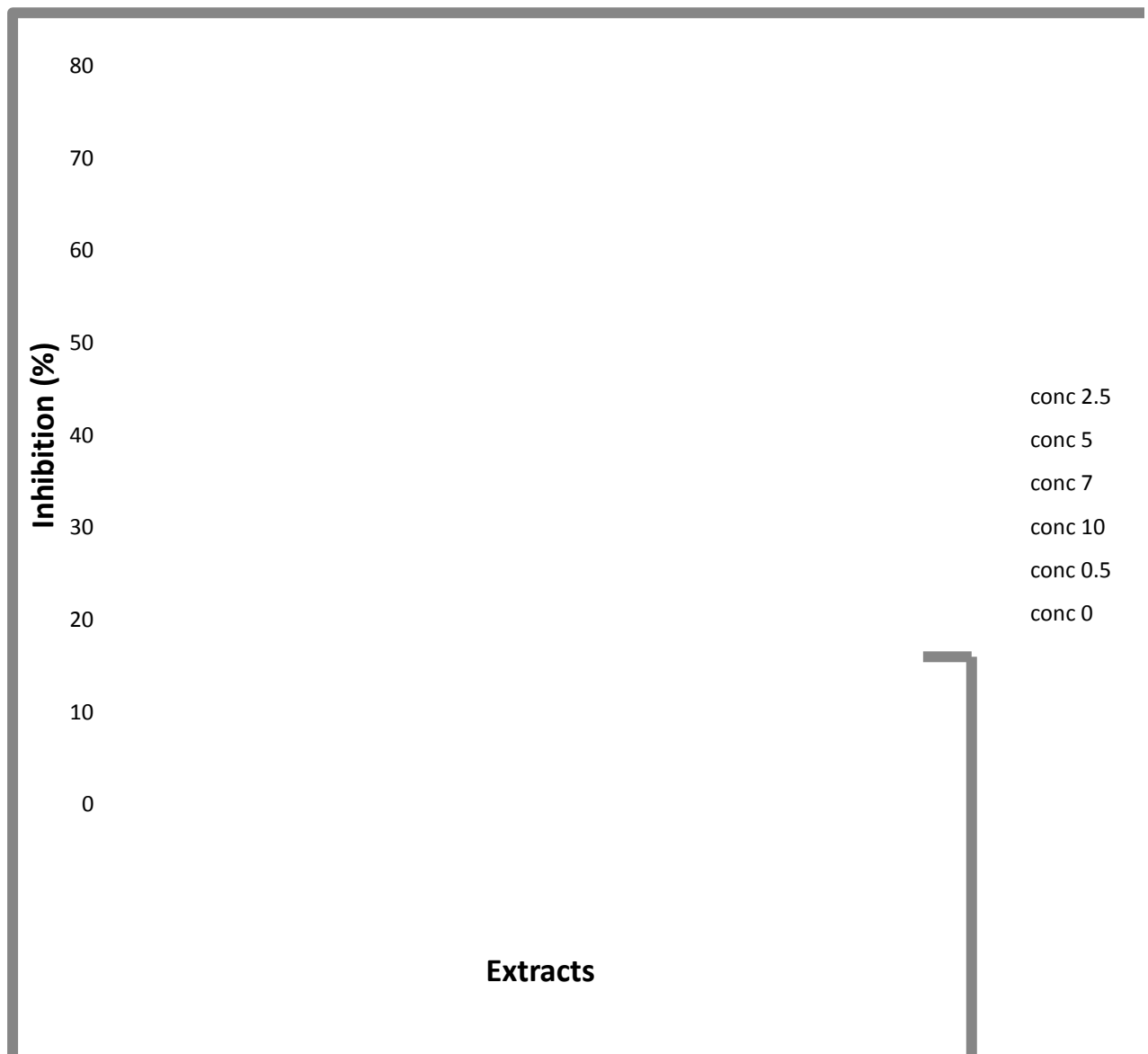
Against *F. solani*, ethanol extract of *A. indica* at 7.5% concentration gave an inhibitory value of 44%, this was significantly ( $P < 0.05$ ) higher than values recorded at other interactions. Ethanol extracts of *O. gratissimum* and *C. citratus* at 7.5 and 10% concentrations gave the next higher values of 42% each, while the next inhibitory value of 38% was observed from Ethanol extract of *V. amygdalina* at 7.5% and 10% concentration, ethanol extract of *A. indica* and ethanol extract of *C. citratus* at 10% concentration each. Aqueous extract were relatively less effective than ethanol extract across all the plant extracts and concentration, hence the least inhibitory effect was recorded from aqueous extract of *O. gratissimum* and aqueous extract of *C. sinensis* at all concentrations (with all the values less than 10%) (Figure 2).



**Figure 2: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Fusarium solani***

**Key:** **ET-Cymbo** means ethanol extract of *Cymbopogon citratus*; **ET-Occimum** means ethanol extract of *Occimum gratissimum*, **ET-Citrus** means ethanol extract of *Citrus sinensis*, **ET-Azadirachta** means ethanol extract of *Azadirachta indica*, **ET-Vernonia** means ethanol extract of *Vernonia amygdalina*, **ET-Carica** means ethanol extract of *Carica papaya*, **AQ-Cymbo** means aqueous extract of *Cymbopogon citratus*; **AQ-Occimum** means aqueous extract of *Occimum gratissimum*, **AQ-Citrus** means aqueous extract of *Citrus sinensis*, **AQ-Azadirachta** means aqueous extract of *Azadirachta indica*, **AQ-Vernonia** means aqueous extract of *Vernonia amygdalina*, **AQ-Carica** means aqueous extract of *Carica papaya*, **P-CTRL-MAN** is Mancozeb (Commercial fungicide),

For *Penicillium* spp, inhibitory values recorded from both aqueous and ethanol extract of *A. indica* and *V. amygdalina* were significantly ( $P<0.05$ ) higher than values obtained from other interactions. Although the highest inhibitory value of 45% was detected from ethanol extract of *A. indica* and *V. amygdalina* at 7.5% concentration each, next to this was 43% value obtained from ethanol extract of *A. indica* at 10% concentration. The least inhibitory values were obtained from aqueous extract of *C. citratus* and ethanol extract of *C. papaya*, while aqueous extract of *C. papaya* did not show any inhibition at all concentrations against *Penicillium* spp. The synthetic fungicide used proved to be more potent by giving an inhibitory zone of 70% (Figure 3).

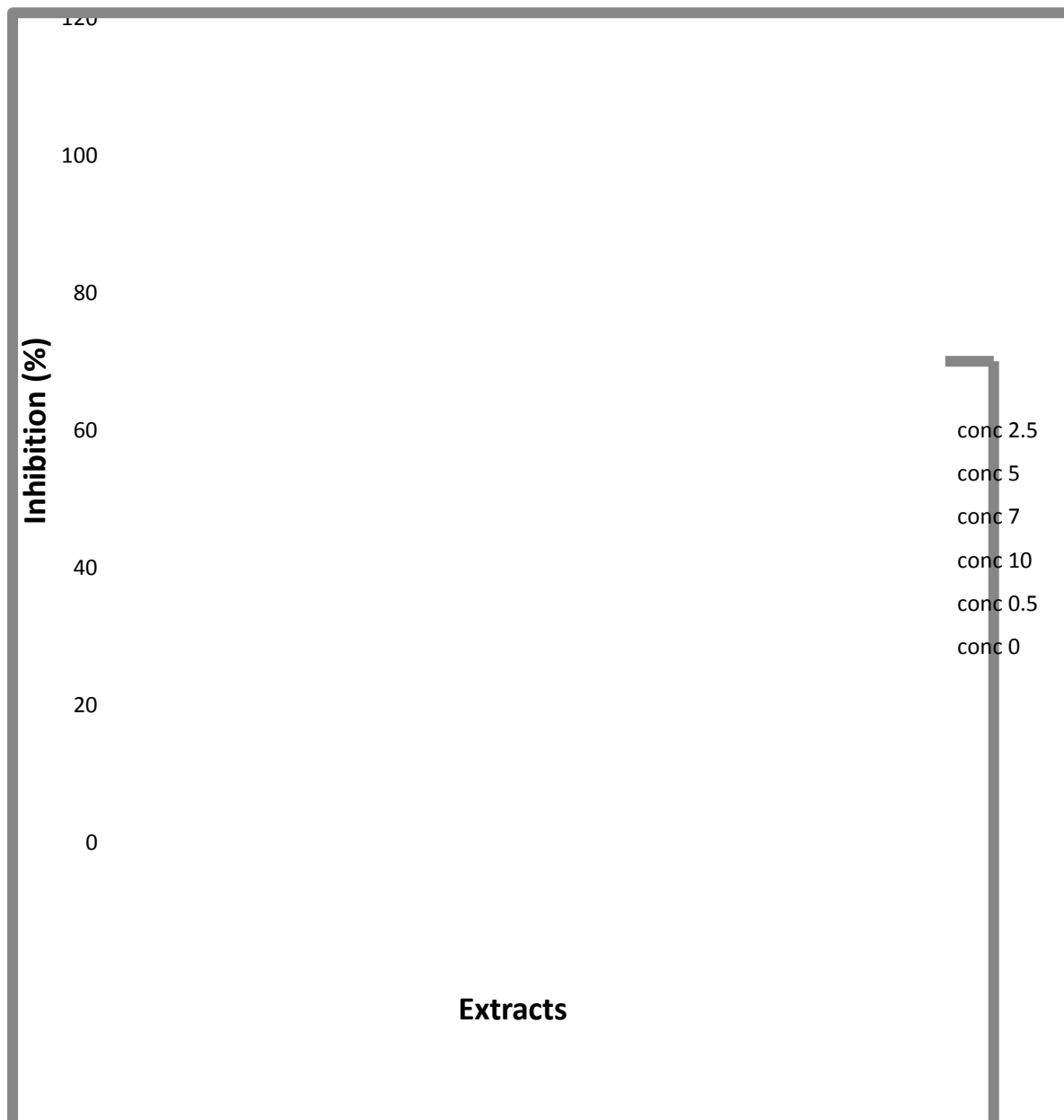


**Figure 3: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Penicillium* spp**

**Key:** **ET-Cymbo** means ethanol extract of *Cymbopogon citratus*; **ET-Occimum** means ethanol extract of *Occimum gratissimum*, **ET-Citrus** means ethanol extract of *Citrus sinensis*, **ET-Azadirachta** means ethanol extract of *Azadirachta indica*, **ET-Vernonia** means ethanol extract of *Vernonia amygdalina*, **ET-Carica** means ethanol extract of *Carica papaya*, **AQ-Cymbo** means aqueous extract of *Cymbopogon citratus*; **AQ-Occimum** means aqueous extract of *Occimum gratissimum*, **AQ-Citrus** means aqueous extract of *Citrus sinensis*, **AQ-Azadirachta** means aqueous extract of *Azadirachta indica*, **AQ-Vernonia** means aqueous extract of *Vernonia amygdalina*, **AQ-Carica** means aqueous extract of *Carica papaya*, **P-CTRL-MAN** is Mancozeb (Commercial fungicide),



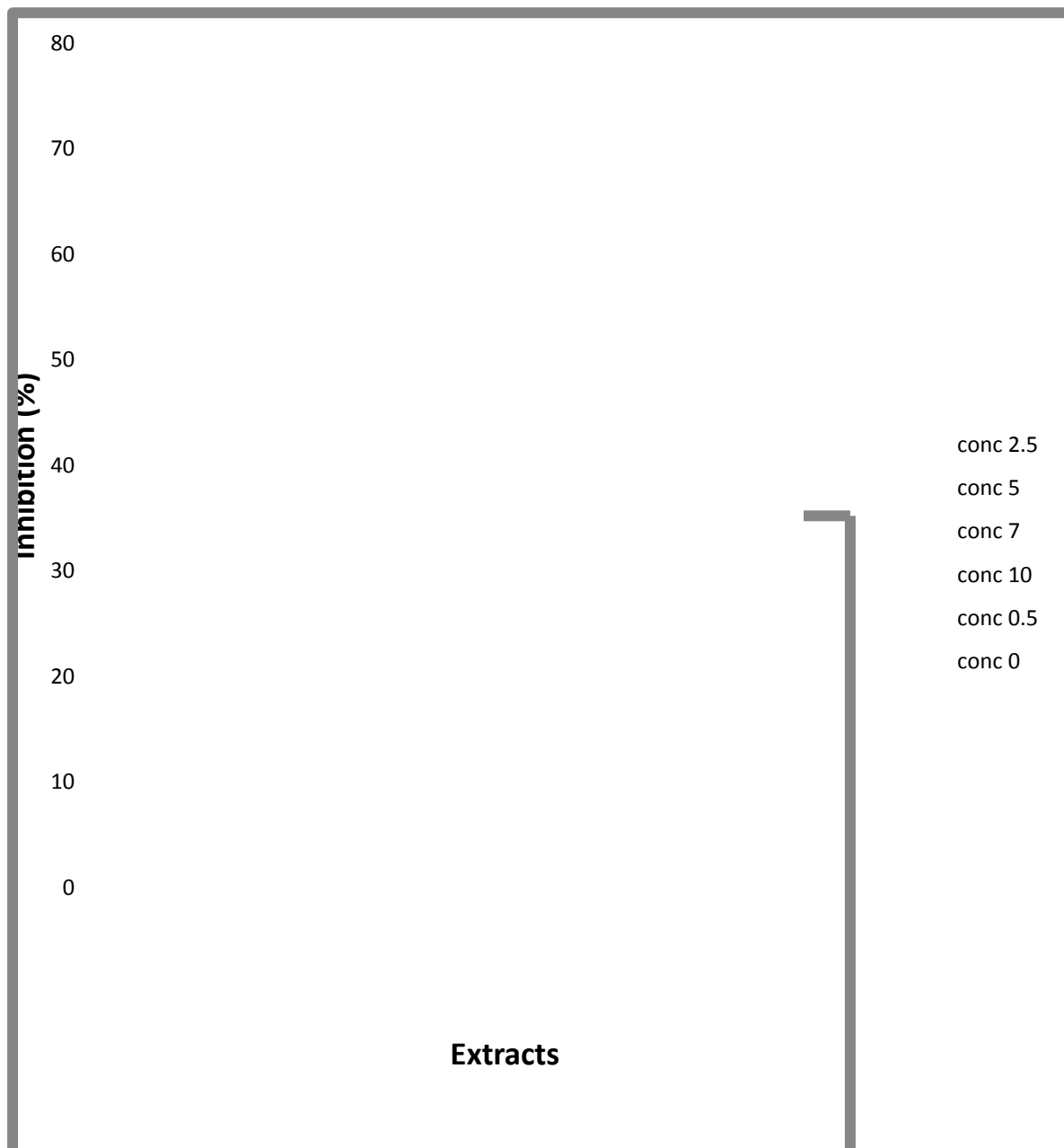
Ethanol extracts of *A.indica* and *V. amygdalina* at 7.5% and 10% concentrations gave the highest inhibitory value (57%) against *A. flavus*, next were ethanol extract of *C. citratus*, ethanol extract of *C. papaya* at 7.5% concentration and ethanol extract of *C. sinensis* at 7.5% and 10% concentrations with value of 42% each. Aqueous extracts showed relatively lower inhibition on *A. flavus*, except *A. indica* and *V. amygdalina* with values of 37% inhibition each. The least inhibition zone was observed from aqueous extracts of *C. sinensis* and aqueous extract of *C. papaya*. The synthetic fungicide used as the positive control gave an inhibitory value of 86%, this was significantly higher than values obtained from other plant materials in all extraction medium at all concentrations (Figure 4).



**Figure 4: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Aspergillus flavus***

**Key:** **ET-Cymbo** means ethanol extract of *Cymbopogon citratus*; **ET-Occimum** means ethanol extract of *Occimum gratissimum*, **ET-Citrus** means ethanol extract of *Citrus sinensis*, **ET-Azadirachta** means ethanol extract of *Azadirachta indica*, **ET-Vernonia** means ethanol extract of *Vernonia amygdalina*, **ET-Carica** means ethanol extract of *Carica papaya*, **AQ-Cymbo** means aqueous extract of *Cymbopogon citratus*; **AQ-Occimum** means aqueous extract of *Occimum gratissimum*, **AQ-Citrus** means aqueous extract of *Citrus sinensis*, **AQ-Azadirachta** means aqueous extract of *Azadirachta indica*, **AQ-Vernonia** means aqueous extract of *Vernonia amygdalina*, **AQ-Carica** means aqueous extract of *Carica papaya*, **P-CTRL-MAN** is Mancozeb (Commercial fungicide),

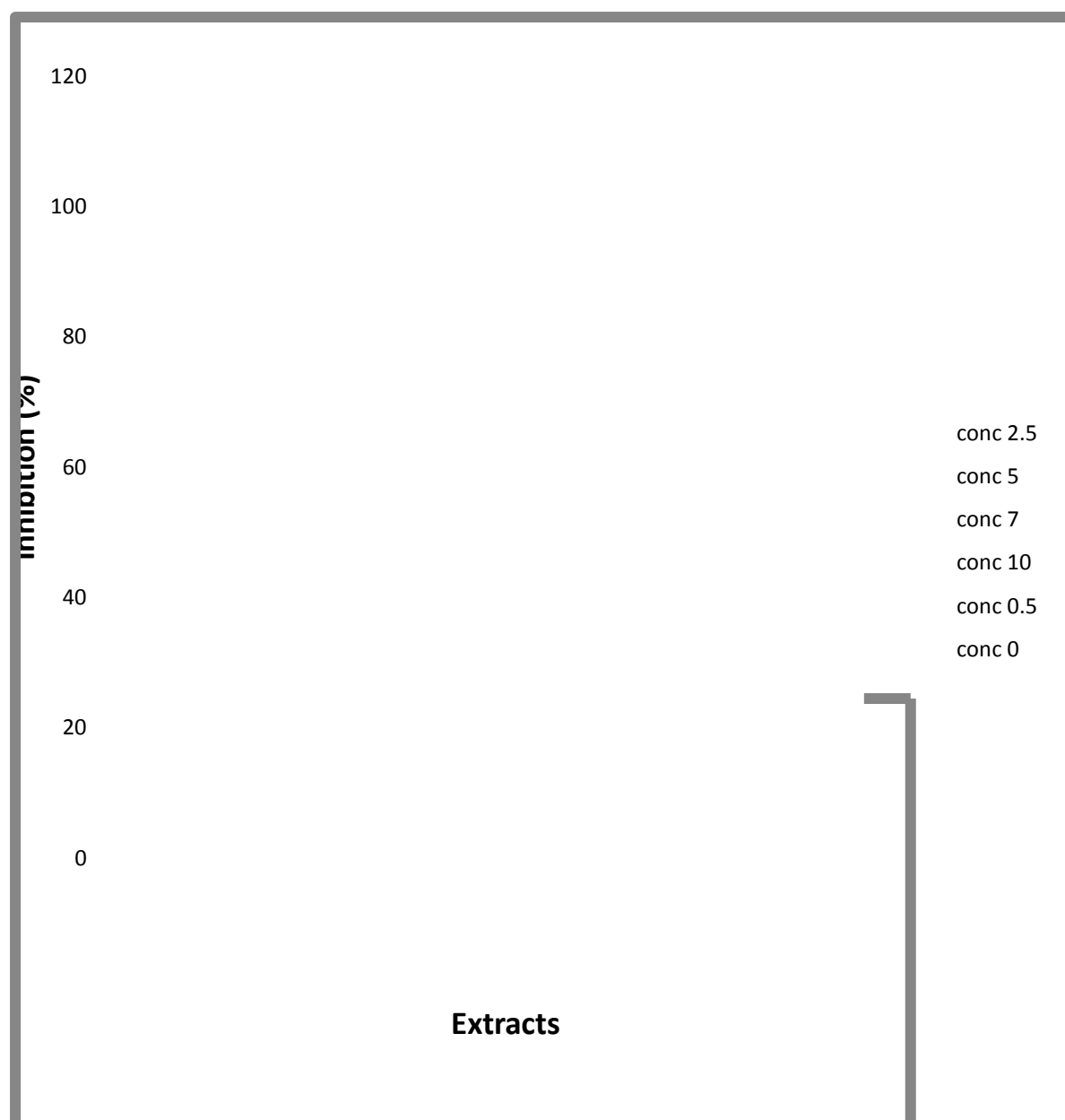
On *F. niger*, ethanol extract of *A. indica* with an inhibitory zone of 63% proved to be more potent than other interaction except the synthetic fungicide used. The next significantly ( $P < 0.05$ ) high value of 55% was obtained from ethanol extract of *V. amygdalina*. Ethanol extract of *A. indica* at 7.5% gave an inhibition zone of 46% while ethanol extract of *C. citratus*, *A. indica* at 5.0% concentration and ethanol extract of *C. papaya* at all concentrations except at 2.5% recorded an inhibition zone of 40%. These values recorded from ethanol extract were significantly higher than values recorded from aqueous extracts. The least inhibitory value was obtained from aqueous extract of *C. citratus* at all concentrations. The synthetic fungicide used gave a value of 67% (Figure 5).



**Figure 5: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Aspergillus niger***

**Key:** **ET-Cymbo** means ethanol extract of *Cymbopogon citratus*; **ET-Occimum** means ethanol extract of *Occimum gratissimum*, **ET-Citrus** means ethanol extract of *Citrus sinensis*, **ET-Azadirachta** means ethanol extract of *Azadirachta indica*, **ET-Vernonia** means ethanol extract of *Vernonia amygdalina*, **ET-Carica** means ethanol extract of *Carica papaya*, **AQ-Cymbo** means aqueous extract of *Cymbopogon citratus*; **AQ-Occimum** means aqueous extract of *Occimum gratissimum*, **AQ-Citrus** means aqueous extract of *Citrus sinensis*, **AQ-Azadirachta** means aqueous extract of *Azadirachta indica*, **AQ-Vernonia** means aqueous extract of *Vernonia amygdalina*, **AQ-Carica** means aqueous extract of *Carica papaya*, **P-CTRL-MAN** is Mancozeb (Commercial fungicide),

For *Botryodiplodia theobromae*, ethanol extracts of *A. indica* gave the highest inhibitory values of 45% at 7.5 and 10% extract concentrations and inhibition zone of 38% at 5.0% concentration. *V. amygdalina* at 7.5% and 10% concentrations gave inhibitory values of 43% and 40% respectively. With respect to aqueous medium, *A. indica* and *V. amygdalina* recorded a relatively higher values of 38% and 36% at 7.5% and 10% concentrations. The least inhibitory value was obtained from aqueous extract of *C. papaya* at 7.5% and 10% concentration, while no inhibition was observed at 2.5% and 5.0%. The inhibition zone of 85% was significantly higher than values obtained from other interactions (Figure 6).



**Figure 6: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Botryodiplodia theobromae***

**Key:** **ET-Cymbo** means ethanol extract of *Cymbopogon citratus*; **ET-Occimum** means ethanol extract of *Occimum gratissimum*, **ET-Citrus** means ethanol extract of *Citrus sinensis*, **ET-Azadirachta** means ethanol extract of *Azadirachta indica*, **ET-Vernonia** means ethanol extract of *Vernonia amygdalina*, **ET-Carica** means ethanol extract of *Carica papaya*, **AQ-Cymbo** means aqueous extract of *Cymbopogon citratus*; **AQ-Occimum** means aqueous extract of *Occimum gratissimum*, **AQ-Citrus** means aqueous extract of *Citrus sinensis*, **AQ-Azadirachta** means aqueous extract of *Azadirachta indica*, **AQ-Vernonia** means aqueous extract of *Vernonia amygdalina*, **AQ-Carica** means aqueous extract of *Carica papaya*, **P-CTRL-MAN** is Mancozeb (Commercial fungicide),

#### **4.5. Synergistic effects of plant extracts on growth of pathogenic organisms**

The investigations as shown in Tables 3 to 8 showed that all the antifungal agents displayed varying degrees of inhibitory effect on the different isolates of the test phytopathogenic fungi spores. Colony diameter of the inhibition increased as the concentration of the extract increased with 7.5% and 10% extract concentrations being more effective than 2.5% and 5.0% extract concentration.

##### **4.5.1. Synergistic Effects of plant extracts on the inhibition of *Fusarium solani***

With respect to aqueous extracts, extract of Neem leaves + Bitter leaves (N+B) gave the highest inhibitory effect of  $94.18 \pm 1.17\%$  this was significantly ( $P < 0.05$ ) higher than the values obtained from the mixture of other plant materials, next to this in terms of inhibitory effect was the mixture of Neem and Lemon (N+L) and Orange + Neem (O+N) which gave the inhibitory percentages of  $74.15 \pm 0.42\%$  and  $76.84 \pm 2.76\%$ , the least inhibitory effect of  $19.18 \pm 0.90\%$  was recorded from the mixture of Scent leaves and bitter leaves, this was significantly lower than the values gotten from the mixture of other plant materials at ( $P < 0.05$ ). For their concentration, 7.5% extract concentration gave the highest inhibitory effect of  $80.82 \pm 1.63\%$ , the least inhibitory effect of  $30.43 \pm 2.33\%$  was recorded from 2.5% extract concentration. For their interaction, mixture of Neem/bitter (N+B) leaves at all the concentrations, mixtures of Orange/Neem (O+N) and Neem/Lemon (N+L) at all the concentrations except at 2.5% extract concentration, had inhibitory percentages ranging between  $92.35 \pm 0.92\%$  and  $96.50 \pm 2.12\%$ , these values were significantly better than values recorded from other interactions. The inhibitory effect was recorded from the mixture of Scent leaves/Bitter leaves (S+B) at all interactions and from Pawpaw and Lemon (P + L) at 2.5% and 5.0% extract concentration (Table 4).

With respect to ethanol extract, the mixtures of Neem/Lemon grass, Bitter leaf/Pawpaw and scent leaf/Bitter leaf were more potent in controlling the growth of *Fusarium solani*, they

gave a high inhibitory percentages of  $94.80\pm 0.46\%$ ,  $96.58\pm 0.39\%$  and  $95.60\pm 0.78\%$  respectively, these were than  $74.26\pm 2.08\%$  recorded from the mixture of Neem/Bitter leaf, the least inhibitory effect was observed from the mixture of Orange/Scent leaf ( $55.05\pm 6.68\%$ ). For concentration, 7.5% extract concentration proved to be more effective, it gave an inhibitory percentage of  $90.01\pm 2.20\%$  next to this was observed from 10% extract concentration while the least inhibitory effect was observed from 2.5% extract concentration with percentage inhibition of  $50.00\pm 0.00\%$ , for their interaction, the mixture of Neem/Lemon, Bitter leaf/Pawpaw and Scent/Bitter leaf at all concentrations were more effective than other interactions, the least inhibitory effect was recorded from the mixture of Orange/Scent leaf at all concentrations except at 2.5% extract concentration where it showed a very high degree of inhibition ( $93.15\pm 0.21\%$ ) (Table 4).



**Table 4: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Fusarium solani*, 7 days after inoculation**

Solvents	Extracts	Concentrations (%)						$\bar{x}$ extract
		0	0.5	2.5	5.0	7.5	10.0	
Aqueous	N+ L			13.10±0.000 <sup>a</sup>	93.50±0.566 <sup>e</sup>	95.40±1.131 <sup>d</sup>	94.60±0.000 <sup>d</sup>	74.15±0.424 <sup>d</sup>
	B + P			16.95±7.283 <sup>b</sup>	28.45±6.859 <sup>b</sup>	94.70±0.424 <sup>d</sup>	45.60±6.223 <sup>b</sup>	47.18±5.195 <sup>b</sup>
	O + N			20.00±0.000 <sup>b</sup>	94.00±1.838 <sup>d</sup>	96.35±0.495 <sup>d</sup>	97.00±0.424 <sup>d</sup>	76.84±2.757 <sup>d</sup>
	S+ B			12.25±0.354 <sup>a</sup>	18.40±0.566 <sup>a</sup>	18.40±0.566 <sup>a</sup>	27.65±5.162 <sup>a</sup>	19.18±0.902 <sup>a</sup>
	N + B			92.35±0.919 <sup>d</sup>	93.25±1.061 <sup>d</sup>	94.60±0.566 <sup>d</sup>	96.50±2.121 <sup>d</sup>	94.18±1.167 <sup>e</sup>
	O + S			39.99±5.454 <sup>c</sup>	16.65±3.547 <sup>a</sup>	69.99±3.972 <sup>b</sup>	95.00±1.414 <sup>d</sup>	53.91±3.600 <sup>c</sup>
	P + L			18.34±2.355 <sup>b</sup>	18.34±2.355 <sup>b</sup>	96.33±4.243 <sup>d</sup>	94.55±1.768 <sup>d</sup>	56.89±2.680 <sup>c</sup>
	NCNTRL	0.00±0.00 0 <sup>a</sup>						
	PCNTRL		96.33±5.49 1 <sup>a</sup>					
	$\bar{x}$ Conc.			30.43±2.337 <sup>c</sup>	51.80±2.399 <sup>c</sup>	80.82±1.628 <sup>c</sup>	78.70±2.444 <sup>c</sup>	
Ethanol	N+ L			97.90±0.283 <sup>e</sup>	98.30±0.283 <sup>d</sup>	91.50±0.707 <sup>c</sup>	91.50±0.707 <sup>d</sup>	94.80±0.495 <sup>d</sup>
	B + P			95.85±0.495 <sup>e</sup>	95.85±0.495 <sup>d</sup>	97.10±0.283 <sup>c</sup>	97.50±0.283 <sup>d</sup>	96.58±0.389 <sup>d</sup>
	O + N			31.65±2.333 <sup>b</sup>	47.20±3.960 <sup>b</sup>	98.40±0.141 <sup>c</sup>	94.70±0.424 <sup>d</sup>	67.99±1.715 <sup>b</sup>
	S+ B			95.10±0.141 <sup>e</sup>	95.10±0.141 <sup>d</sup>	95.10±0.141 <sup>c</sup>	97.10±2.687 <sup>d</sup>	95.60±0.777 <sup>d</sup>
	N + B			8.35±1.809 <sup>a</sup>	94.15±2.333 <sup>d</sup>	97.90±2.970 <sup>c</sup>	96.65±1.202 <sup>d</sup>	74.26±2.078 <sup>c</sup>
	O + S			93.15±0.212 <sup>e</sup>	35.40±2.970 <sup>a</sup>	68.75±8.839 <sup>a</sup>	22.9±14.708 <sup>a</sup>	55.05±6.682 <sup>a</sup>
	P + L			50.00±0.000 <sup>c</sup>	71.65±6.476 <sup>c</sup>	81.65±2.333 <sup>b</sup>	75.00±2.213 <sup>b</sup>	69.58±2.756 <sup>b</sup>
	NCNTRL	0.00±0.00 0 <sup>a</sup>						
	PCNTRL		76.62±1.11 9 <sup>a</sup>					
	$\bar{x}$ Conc			67.43±0.753 <sup>d</sup>	76.81±1.814 <sup>c</sup>	90.01±2.202 <sup>c</sup>	82.19±3.175 <sup>c</sup>	

**Key:** NCNTRL is control,  
 PCNTRL Synthetic fungicide,  
 N+L means mixture of Neem leaves and Lemon grass leaves  
 B+P means mixture of Bitter leaves and Pawpaw leaves  
 O+N means mixture of Orange bark and Neem leaves  
 S+B means mixture of Scent leaves and Bitter leaves  
 N+B means mixture of Neem leaves and Bitter leaves  
 O+S means mixture of Orange bark and Scent leaves  
 P+L means mixture of Pawpaw leaf and Lemon grass leaves

#### 4.5.2. Synergistic Effects of plant extracts on the inhibition of *Rhizopus stolonifer*

With respect to the aqueous extracts, the mixture of Pawpaw/Lemon gave a relatively higher inhibitory percentage of  $34.38 \pm 2.82\%$ , next to this is the mixture of Neem/Bitter leaf ( $10.45 \pm 2.80\%$ ), mixtures of Bitter leaf/Pawpaw, Scent leaf/Bitter leaf and Orange/Scent leaf did not show any inhibition on *Rhizopus*. For concentration, 10% extract concentration proved to be more potent ( $10.87 \pm 2.31\%$ ) while the least inhibitory effect was observed at 2.5% extract concentration ( $1.914 \pm 0.18\%$ ), for their interaction, the mixture of Orange/Scent leaf did not show any inhibition at all the concentrations, the mixture of Neem/Lemon and Orange/Neem did not inhibit the growth of *Rhizopus* at all the concentrations except at 10% extract concentration with values  $1.25 \pm 1.77\%$  and  $8.75 \pm 1.77\%$  respectively, the mixture of Pawpaw/Lemon gave a relatively higher rate of inhibition across all the concentrations (Table 5).

With respect to ethanol extracts, the mixture of Scent leaf/Bitter leaf inhibited the growth of *Rhizopus stolonifer* most with inhibition percentage of  $81.74 \pm 0.02\%$ , this was significantly ( $P < 0.05$ ) higher than  $70.34 \pm 2.67\%$  and  $77.92 \pm 0.02\%$  observed from the mixture of Bitter leaf/Pawpaw and Pawpaw/Lemon respectively, the least inhibitory effect was recorded from the mixture of Orange/Neem and Neem /Bitter leaf with inhibitory values of  $30.31 \pm 1.26\%$  and  $37.66 \pm 6.25\%$  respectively. For concentration, the highest inhibitory effects were recorded at 7.5% and 10% extract concentration while the least values were observed at 2.5% extract concentration ( $34.46 \pm 2.79\%$ ). For their interaction, mixture of Pawpaw/Bitter leaf and mixture of Scent leaf/Bitter leaf all at 7.5% and 10% extract concentration with values ranging from  $99.00 \pm 0.00\%$  to  $99.80 \pm 0.00\%$  proved to be more potent than other interactions, the least inhibitory effect was recorded from the mixture of Neem/Bitter leaf at 2.5% extract concentration  $19.75 \pm 7.43\%$  while the mixture of Orange/Neem at 2.5% extract concentration did not inhibit the growth of *Rhizopus stolonifer* (Table 5).

**Table 5: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Rhizopus stolonifer*, 7 days after inoculation**

Solvents	Extracts	Concentrations (%)						
		0	0.5	2.5	5.0	7.5	10.0	$\bar{x}$ extract
Aqueous	N+ L			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	1.25±1.768 <sup>b</sup>	0.31±0.442 <sup>b</sup>
	B + P			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>
	O + N			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	8.75±1.768 <sup>c</sup>	2.19±0.442 <sup>c</sup>
	S+ B			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>
	N + B			0.00±0.000 <sup>a</sup>	13.40±1.273 <sup>c</sup>	8.75±2.374 <sup>b</sup>	19.65±7.566 <sup>d</sup>	10.45±2.83 <sup>d</sup>
	O + S			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>
	P + L			13.40±1.266 <sup>c</sup>	35.54±3.850 <sup>d</sup>	42.15±1.109 <sup>c</sup>	46.43±5.049 <sup>e</sup>	34.38±2.89 <sup>e</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		41.01±3.107 <sup>a</sup>					
	$\bar{x}$ Conc.			1.914±0.180 <sup>b</sup>	6.991±0.732 <sup>b</sup>	7.271±0.498 <sup>b</sup>	10.87±2.307 <sup>c</sup>	
Ethanol	N+ L			21.25±5.303 <sup>b</sup>	37.50±0.000 <sup>c</sup>	51.25±1.768 <sup>b</sup>	56.25±1.768 <sup>b</sup>	41.56±2.20 <sup>b</sup>
	B + P			40.00±3.536 <sup>c</sup>	42.55±7.142 <sup>c</sup>	99.00±0.000 <sup>e</sup>	99.80±0.000 <sup>e</sup>	70.34±2.60 <sup>c</sup>
	O + N			0.00±0.000 <sup>a</sup>	15.00±3.536 <sup>a</sup>	42.50±3.536 <sup>a</sup>	63.75±1.768 <sup>c</sup>	30.31±1.26 <sup>a</sup>
	S+ B			62.45±0.071 <sup>d</sup>	67.50±0.000 <sup>d</sup>	98.00±0.000 <sup>e</sup>	99.00±0.000 <sup>e</sup>	81.74±0.01 <sup>d</sup>
	N + B			19.75±7.425 <sup>b</sup>	25.90±6.405 <sup>b</sup>	43.55±9.122 <sup>a</sup>	61.45±2.051 <sup>c</sup>	37.66±6.25 <sup>a</sup>
	O + S			35.25±3.182 <sup>c</sup>	35.25±3.182 <sup>c</sup>	68.70±8.910 <sup>c</sup>	22.9±14.708 <sup>a</sup>	40.53±7.49 <sup>b</sup>
	P + L			62.50±0.000 <sup>d</sup>	75.00±0.000 <sup>e</sup>	75.00±0.000 <sup>d</sup>	99.19±0.092 <sup>e</sup>	77.92±0.02 <sup>c</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		37.36±4.200 <sup>a</sup>					
	$\bar{x}$ Conc			34.46±2.788 <sup>c</sup>	42.67±2.895 <sup>c</sup>	68.29±3.334 <sup>c</sup>	71.76±2.912 <sup>d</sup>	

**Key:** NCNTRL is control,  
 PCNTRL Synthetic fungicide,  
 N+L means mixture of Neem leaves and Lemon grass leaves  
 B+P means mixture of Bitter leaves and Pawpaw leaves  
 O+N means mixture of Orange bark and Neem leaves  
 S+B means mixture of Scent leaves and Bitter leaves  
 N+B means mixture of Neem leaves and Bitter leaves  
 O+S means mixture of Orange bark and Scent leaves  
 P+L means mixture of Pawpaw leaf and Lemon grass leaves

#### 4.5.3. Synergistic Effects of plant extracts on the inhibition of *Botrydioplodia theobromae*,

With respect to the effects of aqueous extracts of plant materials on the inhibition of *Botrydioplodia theobromae*, the highest inhibitory percentages were recorded from the mixture of Neem/Bitter leaf and Orange/Scent leaf with values of  $98.40 \pm 0.10\%$  and  $94.24 \pm 0.58\%$  respectively, these values were significantly ( $P < 0.05$ ) better than the mixture of other plant materials, the mixture of Scent leaf/Bitter leaf did not inhibit the growth of *Botrydioplodia theobromae*, while the mixture of Bitter leaf/Pawpaw and Pawpaw/Lemon showed a very slight inhibition with values of  $3.125 \pm 0.00\%$  and  $1.56 \pm 0.46\%$  respectively. For concentration, 7.5% extract concentration gave the highest inhibitory value of  $42.26 \pm 0.00\%$  while the least value was recorded from 5% extract concentration. For their interaction, the mixture of Neem/Bitter leaf and Orange/Scent leaf almost inhibited completely the growth of this organism at all concentrations, the mixture of Neem/Lemon did not show any inhibition at 2.5% and 5.0% extract concentration, the mixture of Bitter leaf/Pawpaw did not show any inhibition at all the concentrations except at 10% while the mixture of Pawpaw/Lemon did not show any inhibition at all the concentrations except at 2.5% (Table 6).

With respect to the effects of ethanol extracts of plant materials on the inhibition of *Botrydioplodia theobromae*, the mixture of Scent leaf/Bitter leaf and the mixture of Pawpaw and Lemon showed the highest inhibition on this organism with values of  $99.80 \pm 0.00\%$  and  $98.83 \pm 0.62\%$  respectively, these values were significantly ( $P < 0.05$ ) higher than values gotten from the mixture of other plant materials, the mixture of Neem/Lemon and the mixture of Bitter leaf/Pawpaw also inhibited the growth of this organism at a relatively reasonable value with values of  $88.38 \pm 2.20\%$  and  $84.46 \pm 1.86\%$ , the least inhibitory percentage was recorded from the mixture of Neem/Bitter leaf ( $34.28 \pm 2.32\%$ ), for the effects of concentrations of ethanol extracts on the inhibition of *Botrydioplodia theobromae*, the highest inhibitory value was observed at

7.5% extract concentration with value of  $89.41 \pm 0.31\%$ , the next high value observed was at 10% extract concentration ( $88.55 \pm 0.95\%$ ) while the least inhibition was observed at 2.5% ( $52.89 \pm 1.77\%$ ). For their interaction, the mixture of Pawpaw/Lemon and the mixture of Scent leaf/Bitter leaf at all concentrations showed a very high inhibition ranging from  $99.25 \pm 0.64\%$  and  $99.80 \pm 0.00\%$ , the mixture of Orange/Neem at 2.5% extract concentration did not inhibit the growth of this organism while the mixture of Neem/Bitter leaf at 2.5% and 5.0% inhibition showed the least inhibition with values of  $7.15 \pm 1.112\%$  and  $17.65 \pm 5.49\%$  respectively, the commercial fungicide gave an inhibitory percentage of  $40.18 \pm 7.20$ , this is less than values recorded from most interactions (Table 6).

**Table 6: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Botrydiopodia theobromae*, 7 days after inoculation**

Solvents	Extracts	Concentrations (%)						$\bar{x}$ extract
		0	0.5	2.5	5.0	7.5	10.0	
Aqueous	N+L			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	37.50±0.000 <sup>b</sup>	70.00±0.000 <sup>d</sup>	26.88±0.000 <sup>c</sup>
	B+P			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	12.50±0.000 <sup>b</sup>	3.125±0.000 <sup>b</sup>
	O+N			0.00±0.000 <sup>a</sup>	5.00±0.000 <sup>b</sup>	65.00±0.000 <sup>d</sup>	75.00±0.000 <sup>d</sup>	36.25±0.000 <sup>d</sup>
	S+B			0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>
	N+B			98.75±0.354 <sup>d</sup>	98.85±0.212 <sup>d</sup>	98.00±0.000 <sup>e</sup>	98.00±1.414 <sup>e</sup>	98.40±0.095 <sup>e</sup>
	O+S			93.50±0.707 <sup>d</sup>	93.15±0.212 <sup>d</sup>	95.30±0.000 <sup>e</sup>	95.00±1.414 <sup>e</sup>	94.24±0.583 <sup>e</sup>
	P+L			6.25±1.839 <sup>b</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	0.00±0.000 <sup>a</sup>	1.56±0.460 <sup>b</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		47.06±6.908 <sup>a</sup>					
$\bar{x}$ Conc.			28.36±0.414 <sup>c</sup>	28.14±0.061 <sup>c</sup>	42.26±0.000 <sup>c</sup>	39.36±0.404 <sup>c</sup>		
Ethanol	N+L			70.00±3.536 <sup>e</sup>	85.00±3.536 <sup>f</sup>	98.65±1.626 <sup>d</sup>	99.85±0.071 <sup>d</sup>	88.38±2.192 <sup>c</sup>
	B+P			66.25±5.303 <sup>e</sup>	73.75±1.768 <sup>e</sup>	98.55±0.354 <sup>d</sup>	99.30±0.000 <sup>d</sup>	84.46±1.856 <sup>c</sup>
	O+N			0.00±0.000 <sup>a</sup>	21.25±5.303 <sup>b</sup>	98.00±0.000 <sup>d</sup>	98.70±0.000 <sup>d</sup>	54.48±0.758 <sup>b</sup>
	S+B			99.80±0.000 <sup>f</sup>	99.80±0.000 <sup>g</sup>	99.80±0.000 <sup>d</sup>	99.80±0.000 <sup>d</sup>	99.80±0.000 <sup>d</sup>
	N+B			7.15±1.112 <sup>b</sup>	17.65±5.486 <sup>a</sup>	57.10±0.000 <sup>a</sup>	55.20±2.687 <sup>a</sup>	34.28±2.321 <sup>a</sup>
	O+S			29.3±1.597 <sup>c</sup>	57.55±7.000 <sup>c</sup>	75.00±0.000 <sup>b</sup>	67.75±3.253 <sup>b</sup>	57.40±2.962 <sup>b</sup>
	P+L			97.70±0.849 <sup>f</sup>	98.85±0.778 <sup>g</sup>	99.55±0.212 <sup>d</sup>	99.25±0.636 <sup>d</sup>	98.83±0.619 <sup>d</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		40.18±7.196 <sup>a</sup>					
$\bar{x}$ Conc			52.89±1.771 <sup>d</sup>	64.84±3.410 <sup>d</sup>	89.41±0.313 <sup>c</sup>	88.55±0.949 <sup>c</sup>		

**Key:** NCNTRL is control,  
 PCNTRL Synthetic fungicide,  
 N+L means mixture of Neem leaves and Lemon grass leaves  
 B+P means mixture of Bitter leaves and Pawpaw leaves  
 O+N means mixture of Orange bark and Neem leaves  
 S+B means mixture of Scent leaves and Bitter leaves  
 N+B means mixture of Neem leaves and Bitter leaves  
 O+S means mixture of Orange bark and Scent leaves  
 P+L means mixture of Pawpaw leaf and Lemon grass leaves

#### 4.5.4. Synergistic Effects of plant extracts on the inhibition of *Penicillium spp*,

With respect to the inhibition of aqueous extracts of plant materials on the inhibition of the growth of *Penicillium spp*, the mixtures of Neem/Lemon, Bitter leaf/Pawpaw and Orange/Neem gave a very high inhibitory effects of  $81.34\pm 0.44\%$ ,  $80.09\pm 0.44\%$  and  $80.74\pm 0.12\%$  respectively, these were significantly ( $P < 0.05$ ) higher than values recorded from the mixture of other plant materials, the inhibitory effect was recorded from the mixture of Orange/Scent leaf ( $18.44\pm 5.88\%$ ), for the effects of concentrations, 7.5% and 10% extract concentrations showed the highest inhibition with values of  $70.33\pm 2.50\%$  and  $73.57\pm 1.12\%$  respectively, while the least inhibitory value was observed at 2.5% extract concentration ( $31.88\pm 2.83\%$ ). For their interaction, the mixtures of Neem/Lemon, Bitter leaf/Pawpaw, Orange/Neem and Scent leaf/Bitter leaf depicted a very high inhibition at 7.5% and 10% extract concentration with values ranging between  $96.00\pm 0.00\%$  and  $98.00\pm 0.00\%$ , the least inhibitory effect was observed from the mixture of Orange/Scent leaf at 2.5%, 5.0% and 7.5% extract concentration and from the mixture of Scent leaf/Bitter leaf at 2.5% concentration (Table 7).

For ethanol extracts on the inhibition of *Penicillium spp*, the mixture of Bitter leaf/Pawpaw and the mixture of Scent leaf/Bitter leaf gave a relatively higher inhibitory effect, with values of  $98.53\pm 0.21\%$  and  $90.34\pm 0.87\%$  these values were not significantly different from each other but significantly higher ( $P < 0.05$ ) than values gotten from the mixture of other plant materials, next to this was  $82.21\pm 1.26\%$  recorded from the mixture of Pawpaw/Lemon, while the least inhibition was observed from the mixture of Orange/Scent leaf. For concentrations, 10% extract concentration gave a value that is higher than other concentrations while the least inhibitory value was recorded from 2.5% extract concentration. For their interactions, the mixture of Bitter leaf/Pawpaw at all concentrations proved to be very effective with values ranging from  $99.10\pm 0.14\%$  to  $97.75\pm 0.35\%$ , the mixtures of Scent leaf/Bitter leaf and the

mixture of Pawpaw/Lemon at all concentrations except at 2.5% concentration gave a very high inhibitory effect, the least inhibitory effect was recorded from the mixture of Orange/Neem and the mixture of Orange/Scent leaf all at 2.5% and 5.0% extract concentration with values ranging between  $2.50\pm 3.54\%$  and  $25.00\pm 0.00\%$ . The synthetic fungicide depicted an inhibitory value of  $78.27\pm 3.67\%$ , this is less than values recorded from some interaction (Table 7).



**Table 7: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Penicillium* spp, 7 days after inoculation**

Solvents	Extracts	Concentrations (%)						$\bar{x}$ extract
		0	0.5	2.5	5.0	7.5	10.0	
Aqueous	N+ L			53.75±1.768 <sup>e</sup>	75.00±0.000 <sup>f</sup>	97.80±0.000 <sup>e</sup>	98.80±0.000 <sup>e</sup>	81.34±0.442 <sup>d</sup>
	B + P			53.75±1.768 <sup>e</sup>	70.00±0.000 <sup>f</sup>	97.80±0.000 <sup>e</sup>	98.80±0.000 <sup>e</sup>	80.09±0.442 <sup>d</sup>
	O + N			33.30±0.000 <sup>d</sup>	94.35±0.495 <sup>g</sup>	97.30±0.000 <sup>e</sup>	98.00±0.000 <sup>e</sup>	80.74±0.124 <sup>d</sup>
	S+ B			18.90±3.111 <sup>b</sup>	48.70±1.838 <sup>d</sup>	96.00±0.000 <sup>e</sup>	98.00±0.000 <sup>e</sup>	65.40±1.237 <sup>c</sup>
	N + B			21.67±7.071 <sup>b</sup>	28.30±2.071 <sup>b</sup>	51.65±2.333 <sup>c</sup>	58.35±1.809 <sup>c</sup>	39.99±3.321 <sup>b</sup>
	O + S			15.00±3.536 <sup>a</sup>	18.75±8.839 <sup>a</sup>	18.75±8.839 <sup>a</sup>	21.25±2.303 <sup>a</sup>	18.44±5.878 <sup>a</sup>
	P + L			26.79±2.524 <sup>c</sup>	37.32±0.255 <sup>c</sup>	33.04±6.314 <sup>b</sup>	40.18±3.790 <sup>b</sup>	34.33±3.221 <sup>b</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		82.79±6.319 <sup>a</sup>					
	$\bar{x}$ Conc.			31.88±2.825 <sup>d</sup>	53.20±1.928 <sup>e</sup>	70.33±2.49 <sup>d</sup>	73.57±1.12 <sup>d</sup>	
Ethanol	N+ L			63.35±4.738 <sup>d</sup>	63.35±4.738 <sup>b</sup>	63.35±4.738 <sup>c</sup>	63.35±4.738 <sup>b</sup>	63.35±4.738 <sup>c</sup>
	B + P			97.75±0.354 <sup>f</sup>	98.15±0.212 <sup>d</sup>	99.10±0.141 <sup>e</sup>	99.10±0.141 <sup>d</sup>	98.53±0.212 <sup>f</sup>
	O + N			5.00±0.000 <sup>a</sup>	25.00±0.000 <sup>a</sup>	40.00±3.536 <sup>b</sup>	99.30±0.000 <sup>d</sup>	42.33±0.884 <sup>b</sup>
	S+ B			84.50±1.697 <sup>e</sup>	92.30±0.849 <sup>d</sup>	92.30±0.849 <sup>e</sup>	99.25±0.071 <sup>d</sup>	90.34±0.867 <sup>f</sup>
	N + B			66.05±12.657 <sup>d</sup>	83.75±5.303 <sup>c</sup>	93.75±8.839 <sup>e</sup>	62.85±4.031 <sup>b</sup>	76.60±7.708 <sup>d</sup>
	O + S			2.50±3.536 <sup>a</sup>	23.05±2.758 <sup>a</sup>	29.3±11.597 <sup>a</sup>	57.55±7.000 <sup>b</sup>	28.10±6.222 <sup>a</sup>
	P + L			36.65±4.738 <sup>b</sup>	96.00±0.000 <sup>d</sup>	98.00±0.000 <sup>e</sup>	98.20±0.283 <sup>d</sup>	82.21±1.255 <sup>e</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		78.27±3.662 <sup>a</sup>					
	$\bar{x}$ Conc			50.87±3.960 <sup>c</sup>	68.84±1.980 <sup>b</sup>	73.69±4.243 <sup>d</sup>	91.79±2.323 <sup>d</sup>	

**Key:** NCNTRL is control,  
 PCNTRL Synthetic fungicide,  
 N+L means mixture of Neem leaves and Lemon grass leaves  
 B+P means mixture of Bitter leaves and Pawpaw leaves  
 O+N means mixture of Orange bark and Neem leaves  
 S+B means mixture of Scent leaves and Bitter leaves  
 N+B means mixture of Neem leaves and Bitter leaves  
 O+S means mixture of Orange bark and Scent leaves  
 P+L means mixture of Pawpaw leaf and Lemon grass leaves

#### 4.5.5. Synergistic Effects of plant extracts on the inhibition of *Aspergillus flavus*,

With respect to the effect of aqueous extracts of plant materials on the inhibition of *Aspergillus flavus*, the highest inhibitory effect was recorded from the mixture of Orange/Scent leaf, this gave a value of  $85.25 \pm 0.88\%$  that is significantly ( $P < 0.05$ ) higher than values recorded from the mixture of other plant materials, a relatively lower inhibitory values were recorded from the mixture of Neem/Bitter leaf and the mixture of Orange/Scent leaf with values of  $39.57 \pm 5.92\%$  and  $35.94 \pm 4.00\%$  respectively, for concentration, the highest inhibitory value was  $77.21 \pm 1.17\%$  recorded at 10% extract concentration, next to this was  $53.41 \pm 4.13\%$  observed at 7.5% extract concentration while the least value of  $29.59 \pm 4.26\%$  was recorded at 2.5% concentration. For their interaction, the mixture of Orange/Neem at 7.5% and 10% concentration, and the mixture of Neem/Lemon, Bitter leaf/Pawpaw and the mixture of Pawpaw/Lemon all at 10% extract concentration inhibited almost completely the growth of *Aspergillus flavus*, while the least inhibitory values were observed from the mixture of Pawpaw/Lemon and Neem/Bitter leaf all at 2.5% extract concentration with values of  $10.00 \pm 14.14\%$  and  $19.05 \pm 3.37\%$  respectively. The synthetic fungicide used gave an inhibitory value of  $60.23 \pm 6.71\%$ , this higher than the values recorded from the mixture of all the plant materials at all most all the concentration except at 10% concentration, exception to this is the mixture of Orange/Neem that gave a very high value at all the concentrations (Table 8).

With respect to ethanol extracts of plant materials on the inhibition of *Aspergillus flavus*, mixture of Neem/Lemon, Scent leaf/Bitter leaf and the mixture of Pawpaw/Lemon depicted a very high inhibitory effect of the organism with values ranging between  $93.15 \pm 0.18\%$  and  $97.99 \pm 1.63\%$ , these were significantly ( $P < 0.05$ ) higher than values recorded from the mixture of other organisms, the least inhibitory value was recorded from the mixture of Orange/Scent leaf ( $39.29 \pm 15.0\%$ ), for concentration, 10% and 7.5% extract concentrations gave a very high

inhibitory values of  $90.68 \pm 1.53\%$  and  $91.71 \pm 0.97\%$  respectively, while the least value of  $59.51 \pm 4.90\%$  was observed at 2.5% extract concentration. For their interactions, all the mixture of plant materials at all 10% extract concentration except the mixture of Neem/Bitter leaf inhibited the growth of *Aspergillus flavus* to a very high extent, the mixture of Neem/Lemon at all concentrations also gave a relatively very high inhibition on the growth of the organism, the least inhibitory value was observed from the mixture of Orange/Scent leaf at all concentrations except at 10% extract concentration. A value of  $71.33 \pm 2.08\%$  recorded from the synthetic chemical used was less than the values gotten from some of the interactions (Table 8).

**Table 8: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Aspergillus flavus*, 7 days after inoculation**

Solvents	Extracts	Concentrations (%)						$\bar{x}$ extract
		0	0.5	2.5	5.0	7.5	10.0	
Aqueous	N+ L			18.85±3.041 <sup>b</sup>	46.05±2.333 <sup>c</sup>	37.70±6.223 <sup>a</sup>	98.35±0.071 <sup>d</sup>	50.24±2.917 <sup>b</sup>
	B + P			18.84±3.062 <sup>b</sup>	26.85±6.718 <sup>a</sup>	37.85±1.485 <sup>a</sup>	98.40±0.141 <sup>d</sup>	45.49±2.852 <sup>b</sup>
	O + N			68.75±1.768 <sup>d</sup>	73.75±1.768 <sup>d</sup>	99.00±0.000 <sup>d</sup>	99.50±0.000 <sup>d</sup>	85.25±0.884 <sup>c</sup>
	S+ B			31.65±0.919 <sup>c</sup>	46.75±2.192 <sup>c</sup>	55.05±0.212 <sup>c</sup>	63.40±2.970 <sup>b</sup>	49.21±1.573 <sup>b</sup>
	N + B			19.05±3.366 <sup>b</sup>	30.36±2.524 <sup>b</sup>	50.79±8.980 <sup>c</sup>	58.07±8.796 <sup>b</sup>	39.57±5.917 <sup>a</sup>
	O + S			40.00±3.536 <sup>c</sup>	31.25±8.839 <sup>b</sup>	47.50±3.536 <sup>b</sup>	25.00±0.000 <sup>a</sup>	35.94±3.991 <sup>a</sup>
	P + L			10.00±14.142 <sup>a</sup>	34.00±8.485 <sup>b</sup>	46.00±8.485 <sup>b</sup>	97.75±0.354 <sup>d</sup>	46.94±7.867 <sup>b</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		60.23±6.709 <sup>a</sup>					
$\bar{x}$ Conc.			29.59±4.262 <sup>c</sup>	41.29±4.694 <sup>c</sup>	53.41±4.132 <sup>c</sup>	77.21±1.172 <sup>c</sup>		
Ethanol	N+ L			93.35±0.636 <sup>f</sup>	99.35±0.071 <sup>c</sup>	93.35±0.636 <sup>b</sup>	93.35±0.636 <sup>b</sup>	93.35±0.636 <sup>c</sup>
	B + P			49.05±6.367 <sup>c</sup>	98.05±0.071 <sup>c</sup>	99.00±0.000 <sup>b</sup>	99.00±0.000 <sup>b</sup>	86.28±1.716 <sup>b</sup>
	O + N			48.50±6.589 <sup>c</sup>	98.35±0.495 <sup>c</sup>	99.00±0.000 <sup>b</sup>	99.65±0.071 <sup>b</sup>	86.38±1.789 <sup>b</sup>
	S+ B			89.40±0.707 <sup>e</sup>	94.40±0.000 <sup>c</sup>	94.40±0.000 <sup>b</sup>	94.40±0.000 <sup>b</sup>	93.15±0.177 <sup>c</sup>
	N + B			26.50±2.121 <sup>b</sup>	96.75±1.061 <sup>d</sup>	98.25±0.354 <sup>b</sup>	55.00±7.071 <sup>a</sup>	69.13±2.652 <sup>c</sup>
	O + S			12.50±17.678 <sup>a</sup>	25.00±35.35 <sup>a</sup>	25.00±5.355 <sup>a</sup>	94.65±1.909 <sup>b</sup>	39.29±15.07 <sup>a</sup>
	P + L			97.30±0.141 <sup>f</sup>	97.65±0.071 <sup>c</sup>	98.30±0.424 <sup>b</sup>	98.70±0.990 <sup>b</sup>	97.99±1.626 <sup>c</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		71.33±2.076 <sup>a</sup>					
$\bar{x}$ Conc			59.51±4.891 <sup>d</sup>	87.08±5.303 <sup>b</sup>	91.71±0.967 <sup>b</sup>	90.68±1.525 <sup>b</sup>		

**Key:** NCNTRL is control,  
 PCNTRL Synthetic fungicide,  
 N+L means mixture of Neem leaves and Lemon grass leaves  
 B+P means mixture of Bitter leaves and Pawpaw leaves  
 O+N means mixture of Orange bark and Neem leaves  
 S+B means mixture of Scent leaves and Bitter leaves  
 N+B means mixture of Neem leaves and Bitter leaves  
 O+S means mixture of Orange bark and Scent leaves  
 P+L means mixture of Pawpaw leaf and Lemon grass leaves

#### 4.5.6. Synergistic Effects of plant extracts on the inhibition of *Aspergillus niger*

With respect to the effects of ethanol extracts of plant materials on the inhibition of *Aspergillus niger*, the mixture of Scent leaf/Bitter leaf gave an inhibitory value of  $76.76 \pm 3.93\%$ , this is significantly ( $P < 0.05$ ) higher than values recorded from the mixture of other plant materials, next to this was  $62.31 \pm 5.47\%$  recorded from the mixture of Orange/Neem, while the least value of  $28.60 \pm 5.09\%$  was recorded from the mixture Neem/Bitter leaf. For concentration, 10% and 7.5% extract concentrations gave a high inhibitory value of  $71.85 \pm 2.605\%$  and  $55.16 \pm 1.75\%$  respectively, the least with respect to concentration was  $27.55 \pm 4.72\%$  observed at 2.5% extract concentration. For their interactions, the highest inhibitory value was recorded from the mixture of Orange/Neem, mixture of Orange/Scent leaf and the mixture of Pawpaw/Lemon all at 10% extract concentration and from the mixture of Orange/Neem at 7.5% extract concentration, the least inhibitory value of  $2.71 \pm 3.83\%$  was recorded from the mixture of Bitter leaf/Pawpaw at 2.5% extract concentration. The synthetic fungicide gave a very high inhibitory value of  $93.93 \pm 8.18\%$  (Table 9).

With respect to the effects of ethanol extracts of plant materials on the inhibition of *Aspergillus niger*, the mixture of Scent leaf/Bitter leaf gave the highest inhibitory values of  $93.45 \pm 0.000\%$ , this is significantly higher than values recorded from the mixtures of other plant materials, next to this were  $83.99 \pm 0.65\%$  and  $82.65 \pm 0.18\%$  inhibition observed from the mixture of Orange/Neem and the mixture of Pawpaw/Lemon, the least value of inhibition was  $55.40 \pm 4.45\%$  recorded from the mixture of Orange/Scent leaf, for concentration, 10% and 7.5% extract concentration a very high inhibitory value of  $92.57 \pm 0.585\%$  and  $90.25 \pm 1.181\%$  respectively, while a lower value of  $58.76 \pm 2.66\%$  was observed at 2.5% extract concentration. For their interaction, all the mixture of plant materials at 10% concentration except the mixture of Orange/Scent leaf inhibited the growth of *Aspergillus niger* very well, the mixture of Bitter

leaf/Pawpaw and the mixture of Scent leaf/Bitter leaf showed a very high inhibition at all concentrations with values ranging between  $87.90\pm 0.00\%$  and  $99.65\pm 0.07\%$ . The synthetic fungicide gave a relatively lower value of  $87.11\pm 3.20\%$ , this is lower than values recorded from most interactions (Table 9).

**Table 9: Effects of aqueous and ethanol extracts of plant materials on the inhibition of *Aspergillus niger* 7 days after inoculation**

Solvents	Extracts	Concentrations (%)						
		0	0.5	2.5	5.0	7.5	10.0	$\bar{x}$ extract
Aqueous	N+ L			5.35±0.071 <sup>b</sup>	57.30±3.536 <sup>e</sup>	45.80±0.141 <sup>b</sup>	61.10±1.556 <sup>b</sup>	42.39±1.326 <sup>c</sup>
	B + P			2.71±3.833 <sup>a</sup>	34.70±0.566 <sup>b</sup>	45.81±0.127 <sup>b</sup>	61.10±1.556 <sup>b</sup>	36.08±1.521 <sup>b</sup>
	O + N			25.00±7.071 <sup>d</sup>	30.00±14.14 <sup>b</sup>	96.60±0.141 <sup>e</sup>	97.65±0.495 <sup>d</sup>	62.31±5.462 <sup>e</sup>
	S+ B			59.45±3.323 <sup>f</sup>	63.85±5.445 <sup>e</sup>	65.30±3.394 <sup>d</sup>	73.90±3.536 <sup>c</sup>	76.76±3.925 <sup>f</sup>
	N + B			20.84±5.890 <sup>c</sup>	26.79±2.524 <sup>a</sup>	24.69±5.494 <sup>a</sup>	42.05±6.435 <sup>a</sup>	28.60±5.086 <sup>a</sup>
	O + S			38.08±6.760 <sup>e</sup>	30.94±3.345 <sup>b</sup>	49.35±0.919 <sup>c</sup>	94.85±4.455 <sup>d</sup>	53.31±3.870 <sup>d</sup>
	P + L			41.43±6.060 <sup>e</sup>	45.72±4.038 <sup>d</sup>	58.57±2.022 <sup>d</sup>	97.29±0.205 <sup>d</sup>	60.75±3.081 <sup>e</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		93.93±8.176 <sup>a</sup>					
	$\bar{x}$ Conc.			27.55±4.715 <sup>d</sup>	41.33±4.799 <sup>d</sup>	55.16±1.748 <sup>d</sup>	71.85±2.605 <sup>c</sup>	
Ethanol	N+ L			43.35±4.738 <sup>a</sup>	97.35±0.495 <sup>e</sup>	83.60±0.420 <sup>b</sup>	90.15±0.212 <sup>b</sup>	78.61±1.466 <sup>c</sup>
	B + P			97.60±0.141 <sup>d</sup>	98.55±0.071 <sup>e</sup>	97.55±2.758 <sup>c</sup>	97.55±2.758 <sup>c</sup>	75.81±1.432 <sup>c</sup>
	O + N			40.35±1.485 <sup>a</sup>	96.50±0.424 <sup>e</sup>	99.45±0.636 <sup>c</sup>	99.65±0.071 <sup>c</sup>	83.99±0.654 <sup>d</sup>
	S+ B			87.90±0.000 <sup>d</sup>	93.90±0.000 <sup>e</sup>	96.00±0.000 <sup>c</sup>	96.00±0.000 <sup>c</sup>	93.45±0.000 <sup>e</sup>
	N + B			35.00±2.213 <sup>a</sup>	46.65±18.88 <sup>a</sup>	95.35±1.909 <sup>c</sup>	99.60±0.141 <sup>c</sup>	69.15±5.786 <sup>b</sup>
	O + S			40.40±10.041 <sup>a</sup>	53.55±5.020 <sup>b</sup>	61.45±2.051 <sup>a</sup>	66.21±0.700 <sup>a</sup>	55.40±4.453 <sup>a</sup>
	P + L			66.70±0.000 <sup>b</sup>	66.70±0.000 <sup>c</sup>	98.35±0.495 <sup>c</sup>	98.85±0.212 <sup>c</sup>	82.65±0.177 <sup>d</sup>
	NCNTRL	0.00±0.000 <sup>a</sup>						
	PCNTRL		87.11±3.195 <sup>a</sup>					
	$\bar{x}$ Conc			58.76±2.660 <sup>b</sup>	79.03±3.556 <sup>d</sup>	90.25±1.181 <sup>c</sup>	92.57±0.585 <sup>b</sup>	

**Key:** NCNTRL is control,  
 PCNTRL Synthetic fungicide,  
 N+L means mixture of Neem leaves and Lemon grass leaves  
 B+P means mixture of Bitter leaves and Pawpaw leaves  
 O+N means mixture of Orange bark and Neem leaves  
 S+B means mixture of Scent leaves and Bitter leaves  
 N+B means mixture of Neem leaves and Bitter leaves  
 O+S means mixture of Orange bark and Scent leaves  
 P+L means mixture of Pawpaw leaf and Lemon grass leaves

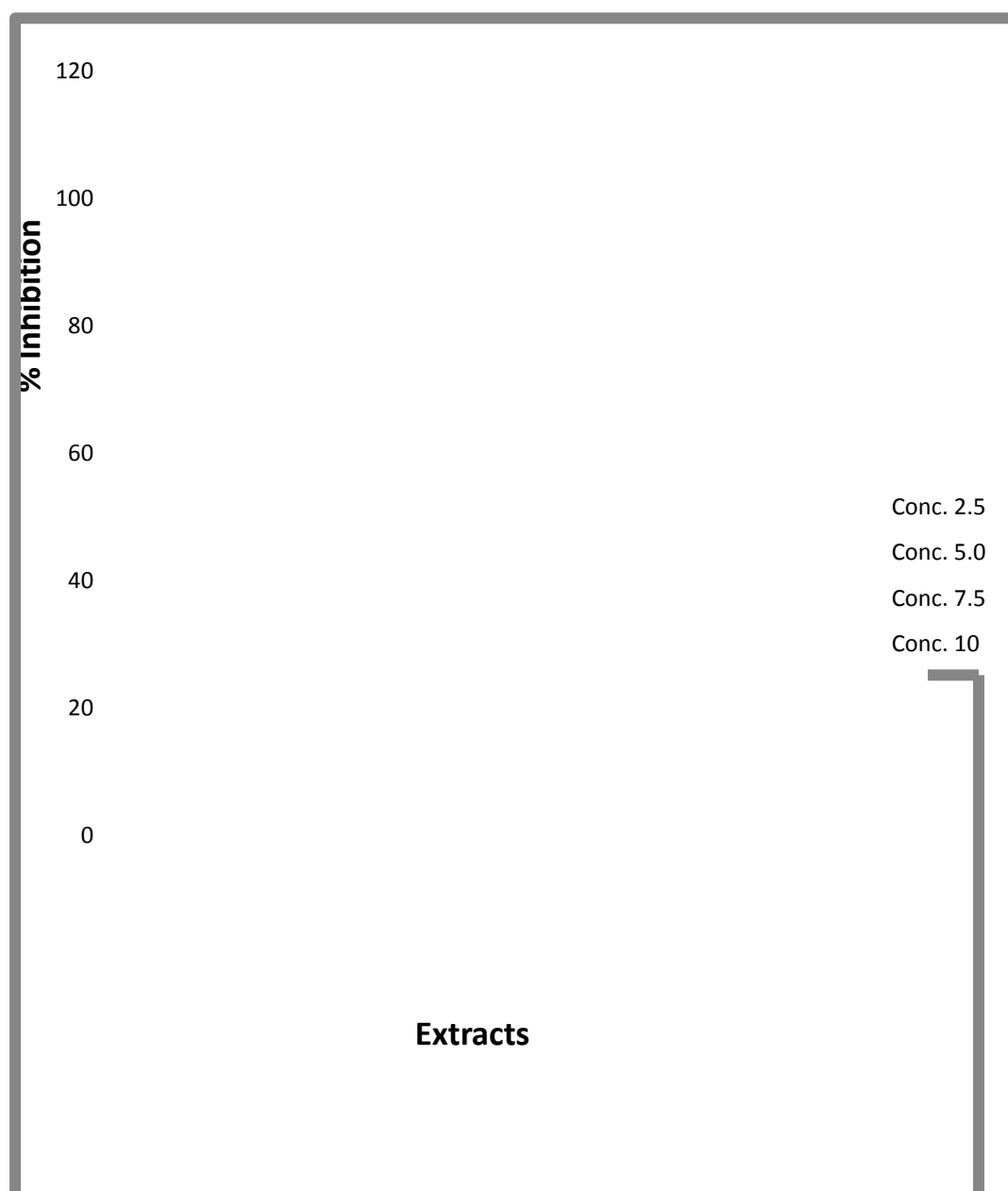
#### **4.6: Comparison between the individual plant extracts and their combination on the inhibition of the test organisms.**

Generally, higher inhibition zones on all the test organisms were recorded from the combined plant extracts than when they were used individually. Hence prominent synergism occurred between plant combinations.

##### **4.6.1. *Aspergillus niger***

With respect to *A. indica* and *C. citratus* on the inhibition of *A. niger*, the values recorded from their combination at all concentrations and in all solvent of extraction proved to be more potent than when used individually. The mixture of *A. indica* and *C. citratus* in ethanol gave an inhibition zones of 94%, 85% and 82% at 5.0%, 10% and 7.5% concentrations, these were significantly higher than values recorded from ethanol extract of *A. indica* an ethanol extract of *C. citratus* (individually) at all concentrations. With respect to aqueous extract, the mixture of *A.indica* and *Ci citratus* at all concentrations except at 2.5% conc. Proved to be more effective in inhibiting the growth of *A. niger* than when they were used separately (Figure 7).

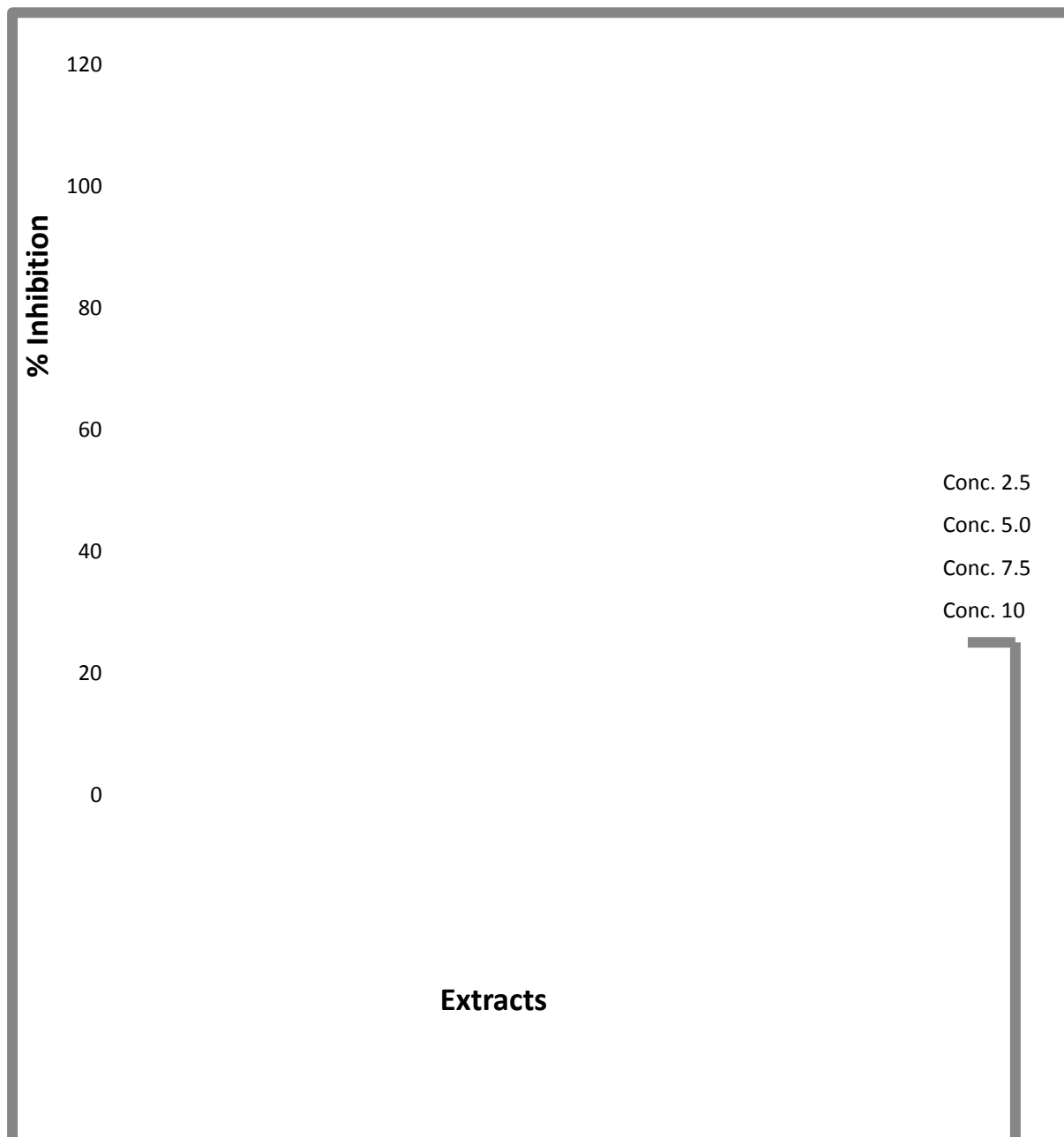




**Figure 7: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Azadi + A-Cymbo** means the mixture of aqueous extract of *Azadirachta indica* and *Cymbopogon citratus*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Cymbo** Means ethanol extract of *Cymbopogon citratus*, **E-Azadi + E-Cymbo** means the mixture of ethanol extract of *Azadirachta indica* and *Cymbopogon citratus*

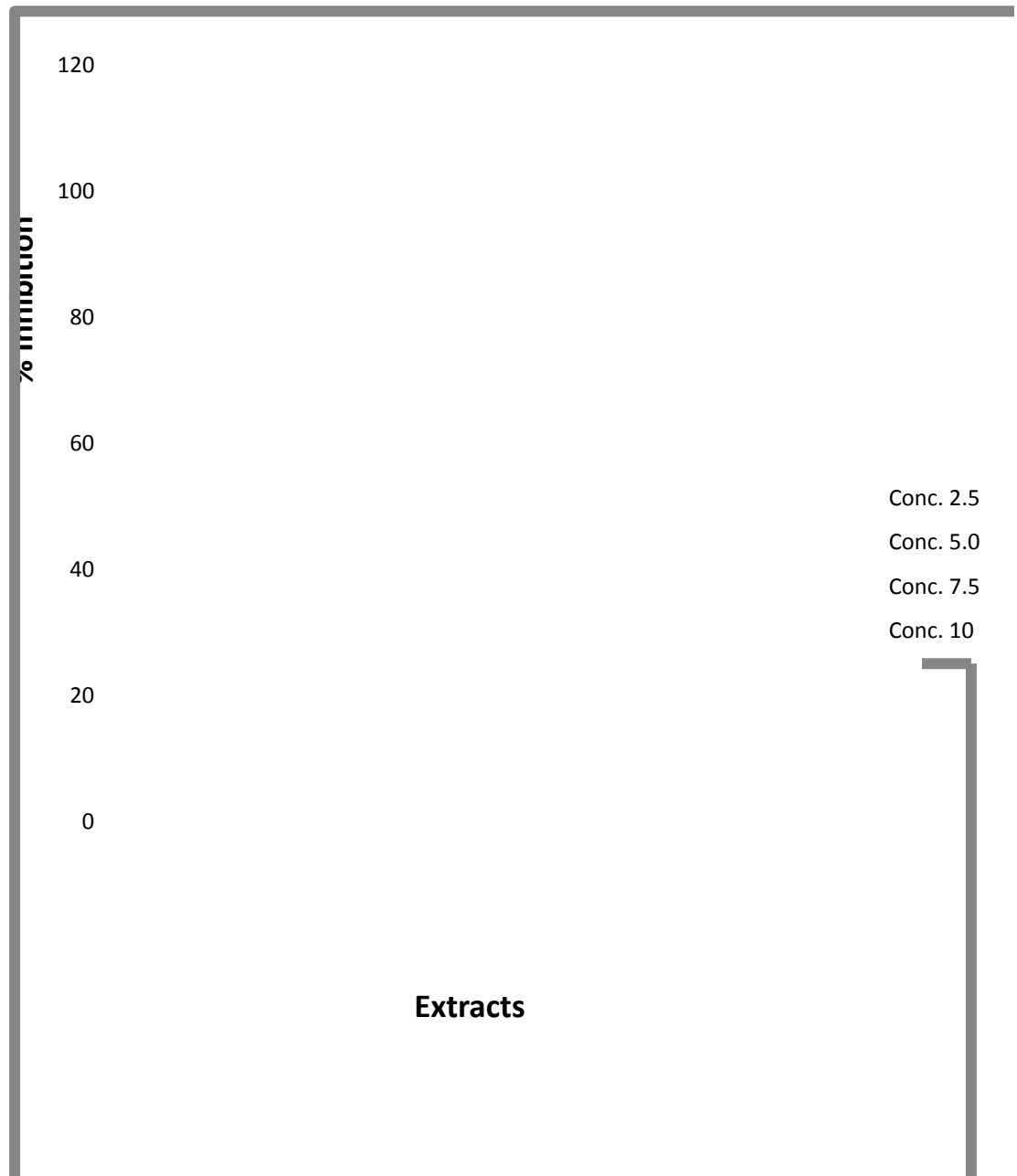
For *V. amygdalina* and *C. papaya* on the inhibition of *A. niger*, the ethanol mixture of *V. amygdalina* and *C. papaya* gave an inhibition zone of 98% at all concentrations this was significantly higher than the values recorded from the individual plant extracts. Ethanol extract of *V. amygdalina* has its highest value of 60% inhibition at 10% concentration while ethanol extract of *C. papaya* has its highest value of 40% inhibition at all the concentration except at 2.5%. With respect to aqueous extract, there was a positive extract combination, with the mixture of *V. amygdalina* and *C. papaya* at all the concentrations except at 2.5% showing inhibition zones of 32%, 44% and 60% at 5.0%, 7.5% and 10% concentrations respectively. The highest value recorded from aqueous extract of *V. amygdalina* was 30% at 7.5% and 10% concentrations each while the highest value obtained from aqueous extract of *C. papaya* was 16% at 7.5% concentration (Figure 8).



**Figure 8: Comparison between aqueous and ethanol extracts of *Vernonia amygdalina* and *Carica papaya* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Vernonia** means aqueous extract of *Vernonia amygdalina*, **A-Carica** Means aqueous extract of *Carica papaya*, **A-Vernonia + A-Carica** means the mixture of aqueous extract of *Vernonia amygdalina* and *Carica papaya*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina*, **E-Carica** Means ethanol extract of *Carica papaya* **E-Vernonia+ E-Carica** means the mixture of ethanol extract of *Vernonia amygdalina* and *Carica papaya*

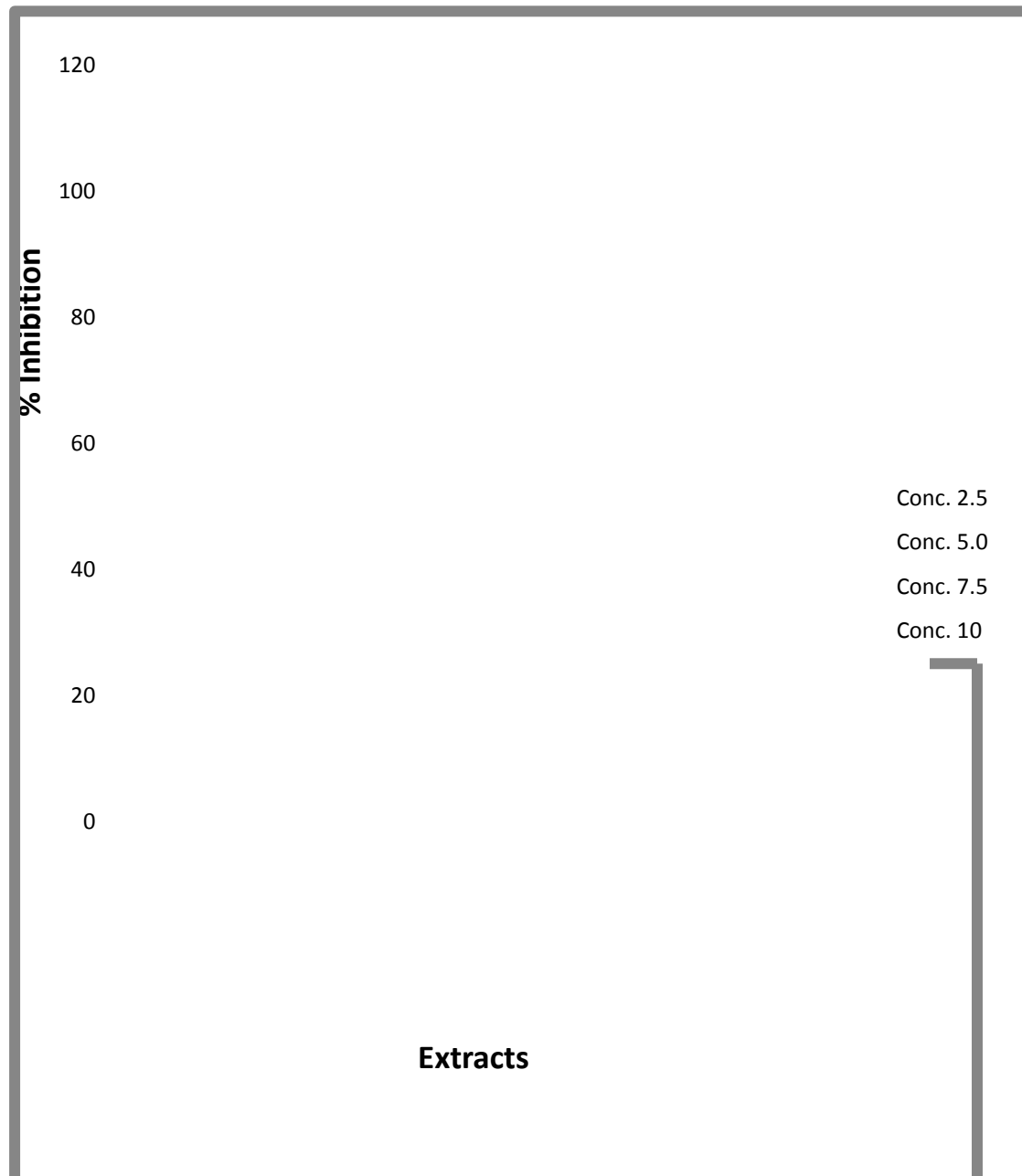
For the comparison between the effects of aqueous and ethanol extract of *C. sinensis* and aqueous and ethanol extract of *A. indica* and their combination on the inhibition of *A. niger*, the combination of *C. sinensis* and *A. indica* in ethanol medium at all the concentrations except at 5.0% concentration gave the highest inhibitory effect of 97%, 99% and 100% at 5.0%, 7.5% and 10% concentrations. Their combination in aqueous at 7.5% and 10% concentrations gave relatively high values of 92% and 94% respectively. These were better than the effects of the individual plant extracts. The highest inhibition zone recorded from the individual plant extract was 62% observed from ethanol extract of *A. indica* at 10% concentration while (Figure 9).



**Figure 9: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Azadirachta indica* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Azadi** Means aqueous extract of *Azadirachta indica*, **A-Citrus + A-Azadi** means the mixture of aqueous extract of *Citrus sinensis* and *Azadirachta indica*, **E-Citrus** means ethanol extract of *Citrus sinensis* **E-Azadi** Means ethanol extract of *Azadirachta indica* **E-Citrus+ E-Azadi** means the mixture of ethanol extract of *Citrus sinensis* and *Azadirachta indica*

For the comparison between the effects of *O. gratissimum*, *V. amygdalina* and their combination on the inhibition of *A.niger*, the mixture of *O. gratissimum* and *V.amygdalina* in ethanol at all concentrations gave a near complete inhibition of the organism with values ranging from 85% to 92% zone of inhibition. These values were far higher than the highest value recorded from the individual plant extract at all concentrations. The combination of *O. gratissimum* and *V. amygdalina* in aqueous recorded a high value of inhibition ranging from 60% to 72%, these were significantly higher than the highest inhibition value of 30% recorded from the aqueous extract of the individual plants (Figure 10).

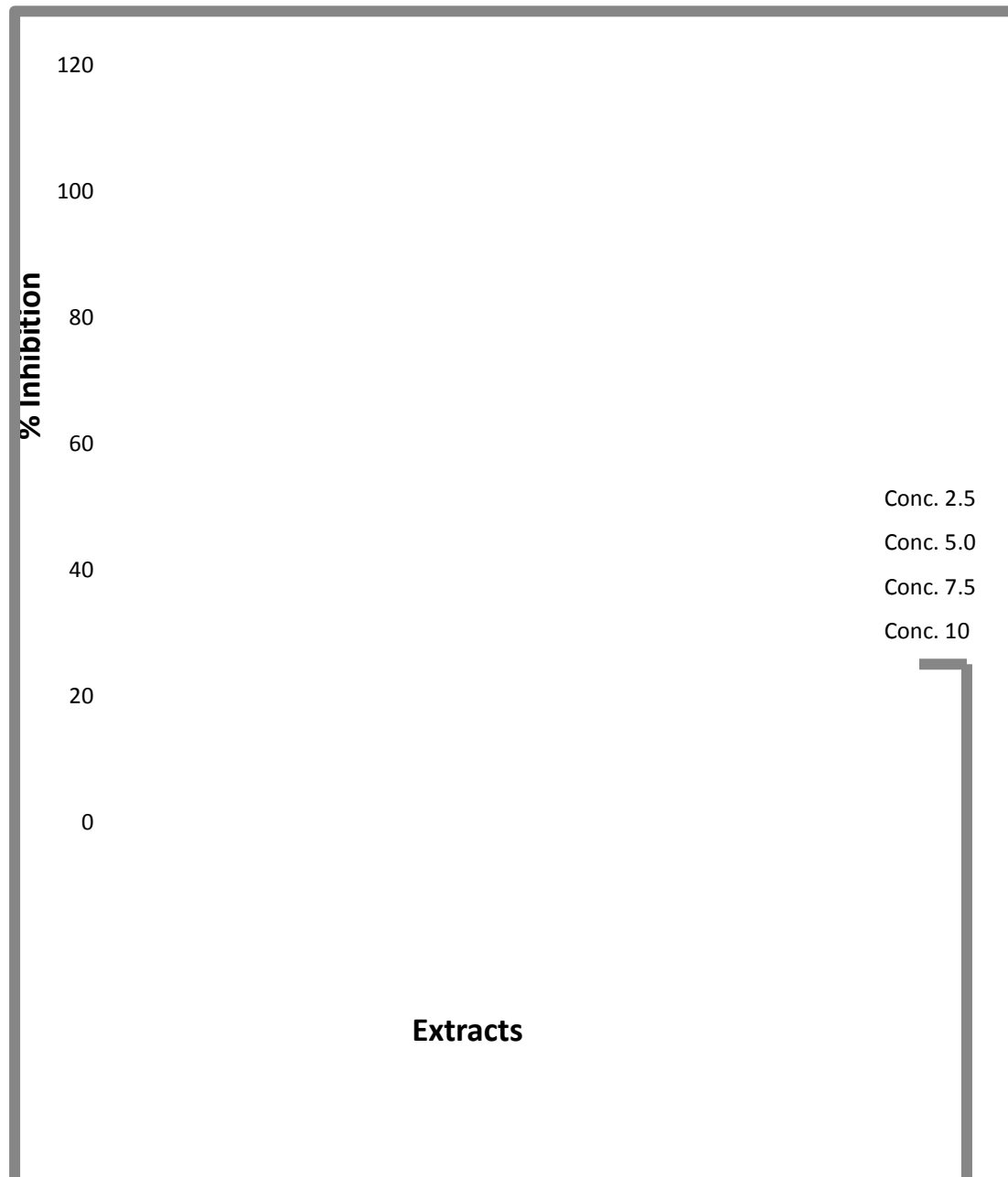


**Figure 10: Comparison between aqueous and ethanol extracts of *Occimum gratissimum* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Occimum + A-Vernonia** means the mixture of aqueous extract of *Occimum grassimum* and *Vernonia amygdalina*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Occimum+ E-Vernonia** means the mixture of ethanol extract of *Occimum gratissimum* and *Vernonia amygdalina*

For the comparison between *A. indica*, *V. amygdalina* and their combination on the inhibition of *A. niger*, with respect to the ethanol medium, there was a prominent synergism between the plant extracts when mixed together. Their combination at 7.5% and 10% concentrations gave inhibition values of 96% and 100% respectively, these were significantly ( $P < 0.05$ ) higher than values recorded from the individual plant extract at 7.5% and 10% concentrations. For aqueous extracts, there was no synergistic effects of the combined plant extracts on *A. niger* the values recorded from the individual plant extracts and the combined plant extracts were almost the same except at 10% concentration where aqueous extract of *A. indica* gave an inhibition value of 40%, while *V. amygdalina* gave 35% and their combination gave 43% (Figure 11).

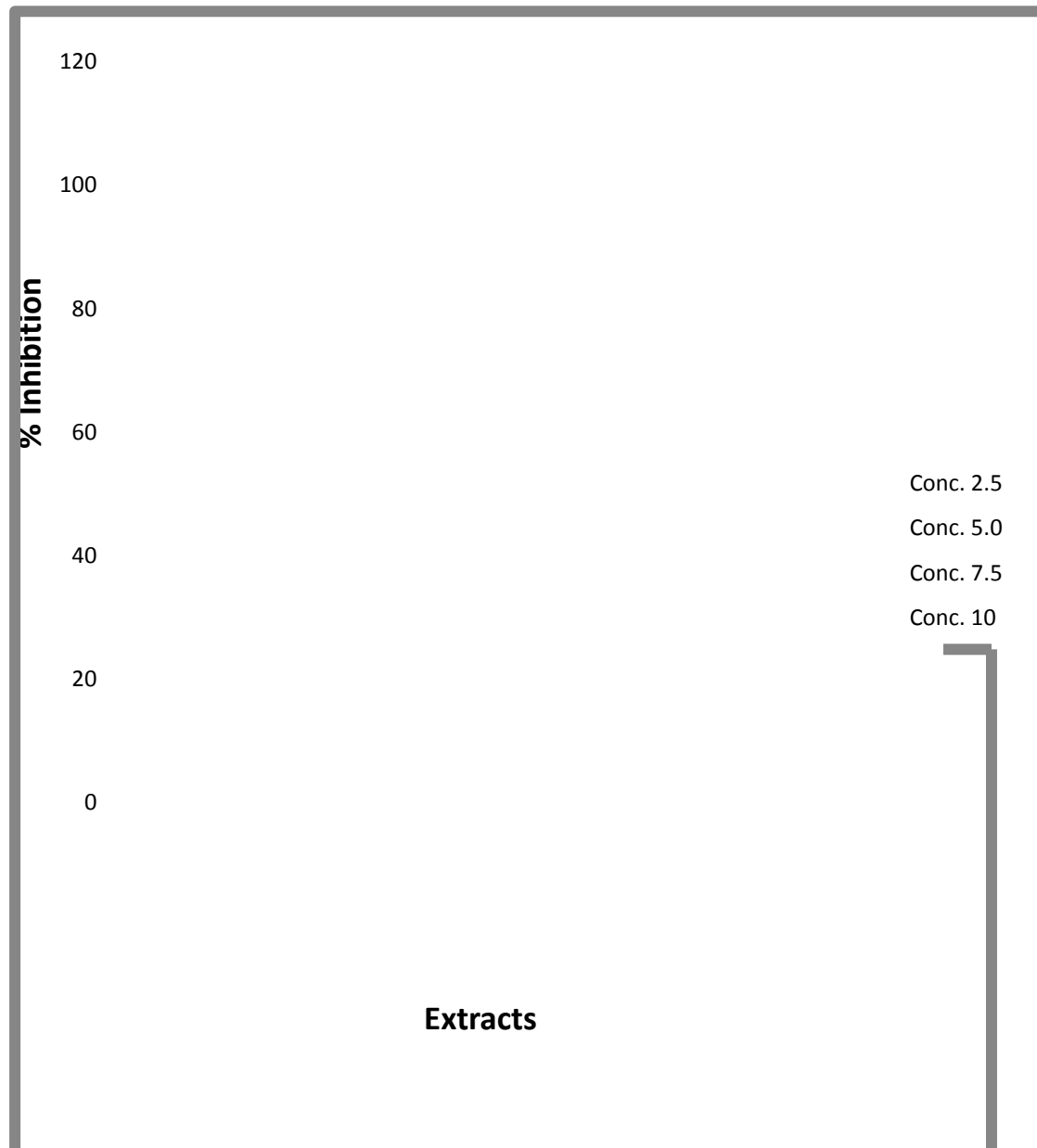




**Figure 11: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Azadi + A-Vernonia** means the mixture of aqueous extract of *Azadirachta indica* and *Vernonia amygdalina*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Azadi+ E-Vernonia** means the mixture of ethanol extract of *Azadirachta indica* and *Vernonia amygdalina*

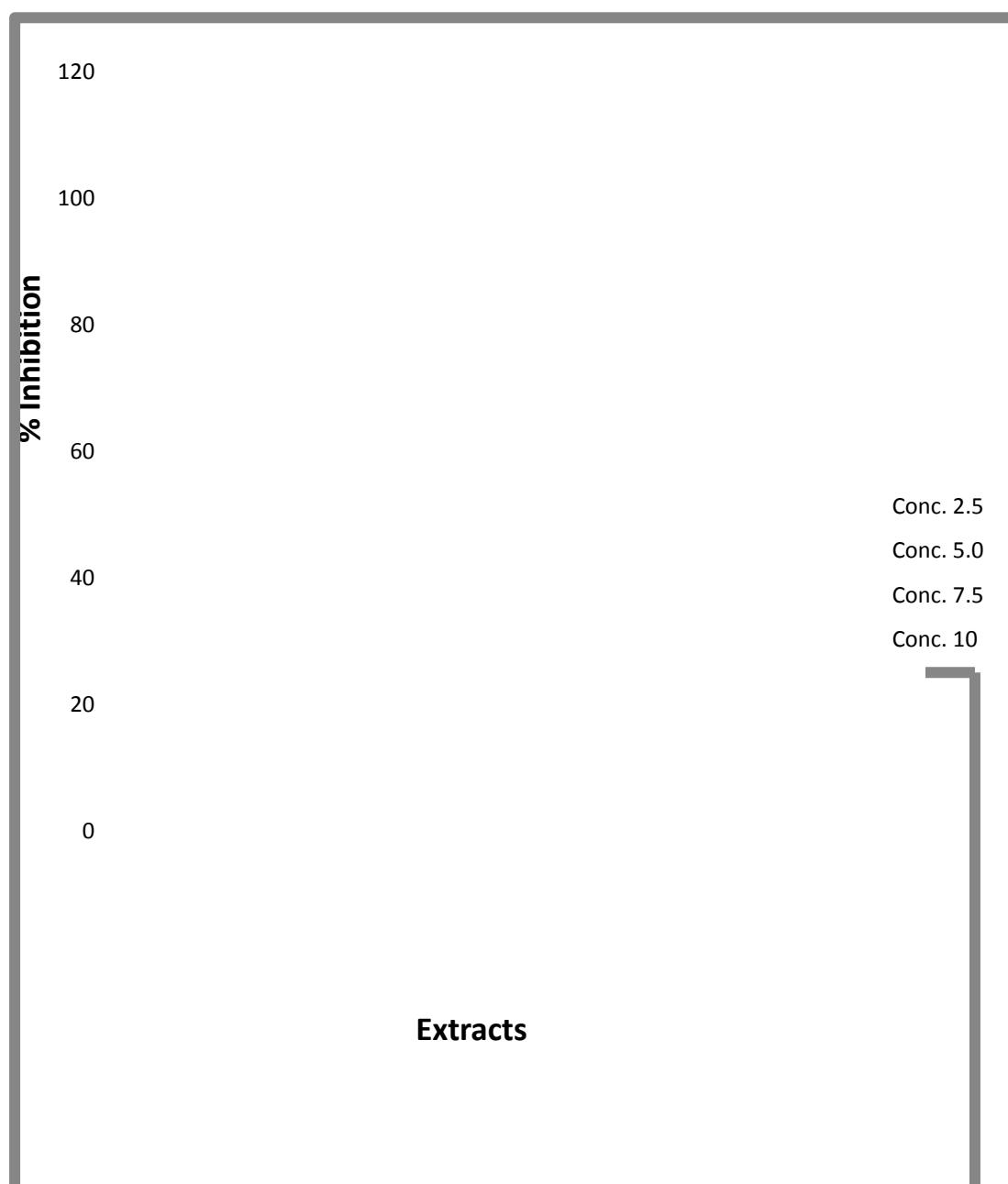
There was synergism in the effects of *C.citratrus* and *O. gratissimum* on the inhibition of *A.niger* at all concentrations. With respect to ethanol medium, the values recorded from their combinations ranged from 65%, 62%, 56% and 40% at 10%, 7.5%, 5% and 2.5% concentrations respectively. These were significantly ( $P<0.05$ ) higher than values recorded from the individual plant extracts. Ethanol extract of *O. gratissimum* gave an inhibition value of 9.5% at all the concentrations while *C. citratrus* gave the highest inhibitory value of 24% at 5.0 and 7.5% concentrations each. For aqueous, the combination of *C. citratrus* and *O. gratissimum* showed a very high synergistic effect on *A. niger* especially at 10% and 7.5% concentrations. At 10% concentration, their combination gave an inhibition value of 92% while *O. gratissimum* gave 18% inhibition and *C. sinensis* gave 4% inhibition (Figure 12).



**Figure 12: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Occimum gratissimum* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-citrus +A-Occimum** means the mixture of aqueous extract of *Citrus sinensis* and *Occimum gratissimum*, **E-Citrus** means ethanol extract of *Citrus sinensis*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Citrus + E-Occimum** means the mixture of ethanol extract of *Citrus sinensis* and *Occimum gratissimum*.

For the comparison between the effects of *C. papaya*, *C. citratus* and their combination on the inhibition of *A. niger*, there was synergistic effects in both aqueous and ethanol media. For ethanol, the combination of *C. papaya* and *C. citratus* gave the highest inhibition at all concentrations, 7.5% and 10% concentrations gave inhibition values of 99% each while 2.5% and 5.0% concentrations gave 68% inhibition values each. These were significantly ( $P < 0.05$ ) higher than 40%, 36% and 10% obtained from *C. citratus* at 10%, 7.5% and 5.0% concentrations respectively. Ethanol, extract of *C. papaya* gave inhibition value of 40% at all concentrations except at 2.5% where no inhibition was observed. For aqueous, the combination of *C. papaya* and *C. citratus* proved to be more potent than the effects of the individual extract, the values recorded from their combination were 99%, 59%, 44% and 40% at 10%, 7.5%, 5% and 2.5% concentrations respectively. Values obtained from the individual plant extracts ranged from 9% to 18% across all the concentrations (Figure 13).

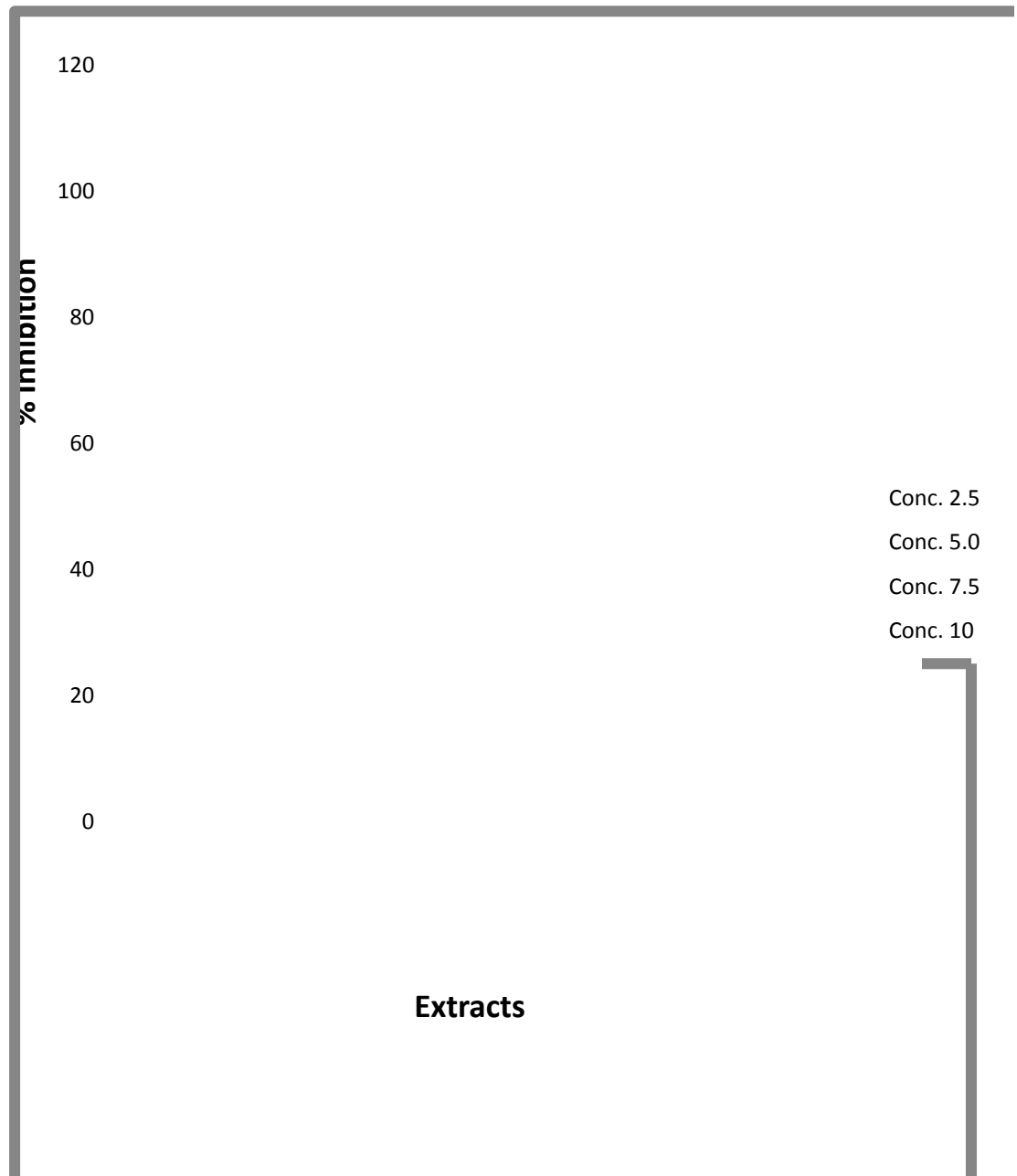


**Figure 13: Comparison between aqueous and ethanol extracts of *Carica papaya* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Aspergillus niger***

Key: **A-Carica** means aqueous extract of *Carica papaya*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Carica + A-Cymbo** means the mixture of aqueous extract of *Carica papaya* and *Cymbopogon citratus*, **E-Carica** means ethanol extract of *Carica papaya*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus* **E-Carica+ E-Cymbo** means the mixture of ethanol extract of *Carica papaya* and *Cymbopogon citratus*

#### 4.6.2. *Penicillium* spp

For the comparison between the effects of *C. sinensis*, *A. indica* and their combination on the inhibition of *Penicillium* Spp, with respect to ethanol the effects of the combined plant extracts was not significantly higher than the effects of the individual plant extract at all concentrations except at 10%. The combination of these extracts at 10% concentration gave a complete inhibition (100%) on the test fungi, these was ( $P < 0.05$ ) significantly higher than 41% recorded from 10% concentration of *A. indica* and 18% recorded from 10% concentration of *C. sinensis*. For aqueous extract, there was high synergism between *C. citratus* and *A. indicia* at all concentrations except at 2.5%, the values recorded from these combination ranged from 92% to 96%. These were significantly higher than the highest value of 37.5% obtained from *A. indica* at 10% concentration and 17.5% from *Citrus sinensis* at 10% concentration (Table 14).

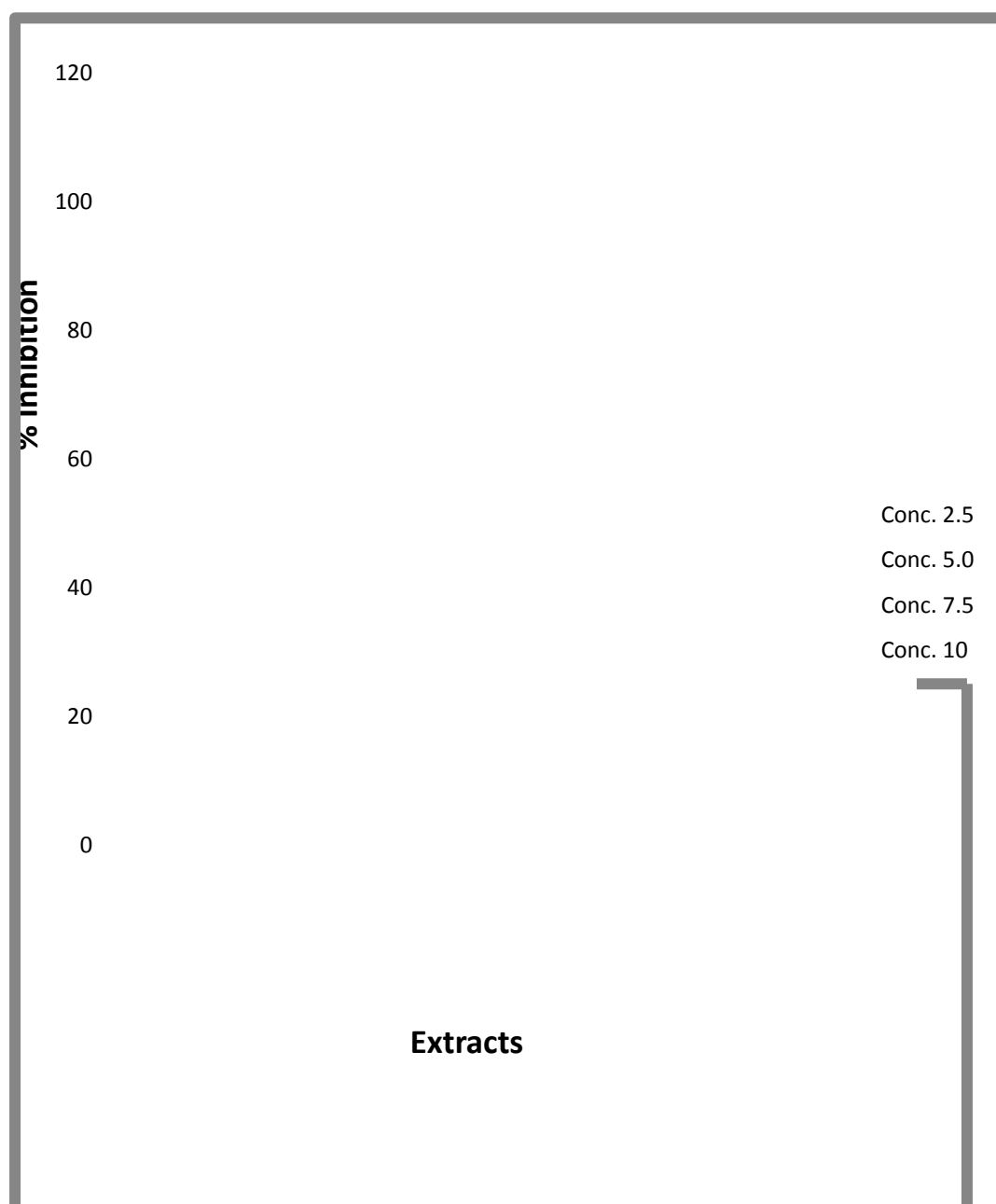


**Figure 14: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Azadirachta indica* and their synergistic effects on the inhibition of *Penicillium* spp**

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Azadi** Means aqueous extract of *Azadirachta indica*, **A-Citrus + A-Azadi** means the mixture of aqueous extract of *Citrus sinensis* and *Azadirachta indica*, **E-Citrus** means ethanol extract of *Citrus sinensis* **E-Azadi** Means ethanol extract of *Azadirachta indica* **E-Citrus+ E-Azadi** means the mixture of ethanol extract of *Citrus sinensis* and *Azadirachta indica*

For *A. indica* and *C. citratus* on *Penicillium* Spp, there was a clear synergism with more inhibition observed when the extracts were combined. For ethanol, the combination of *A. indica* and *C. citratus* gave 60% inhibition across all the concentrations. These were significantly ( $P<0.05$ ) higher than values obtained from each of the individual plant extracts. The highest value of 44% obtained from *A. indica* at 7.5% concentration was lower than the value obtained when the two plant extracts were combined. The values obtained from *C.citratus* ranged between 10 to 22% these were significantly lower than all the values obtained when the two plant extracts were combined. For aqueous, the combination of the two plant extracts at 7.5% and 10% concentrations gave a near complete inhibition (99%) on the test organism, while at 5.0% and 2.5% concentration, an inhibition value of 73% and 52% respectively were recorded. These values were significantly ( $P<0.05$ ) better than values recorded from the individual plant extracts. For the individual plant extracts, the inhibition percentages obtained from the aqueous extract of *A.indica* ranged from 22% to 38% while that of aqueous extract of *C.citratus* ranged from 4% to 9% inhibition (Figure 15).

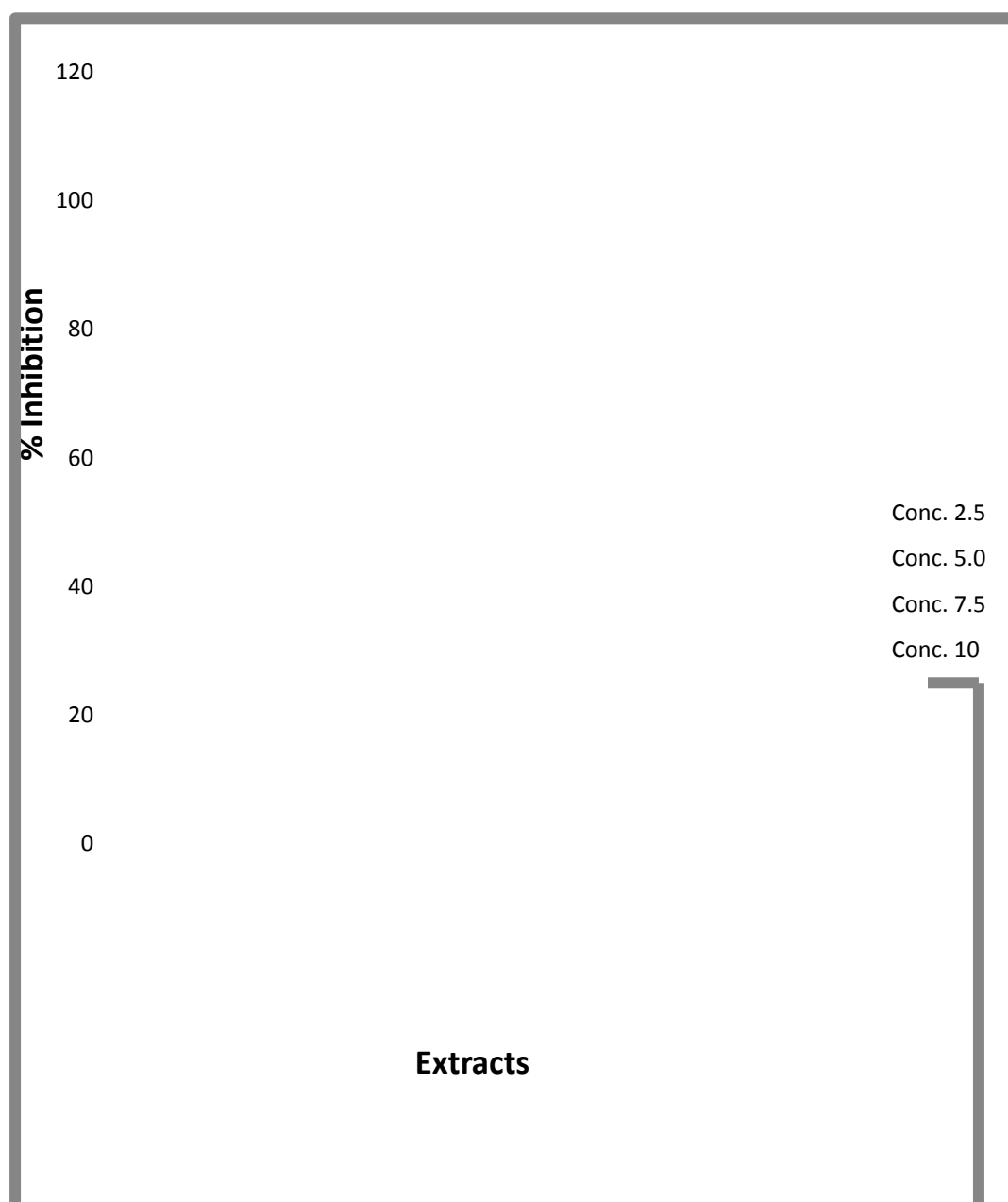




**Figure 15: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Penicillium* spp**

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Azadi + A-Cymbo** means the mixture of aqueous extract of *Azadirachta indica* and *Cymbopogon citratus*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Cymbo** Means ethanol extract of *Cymbopogon citratus*, **E-Azadi + E-Cymbo** means the mixture of ethanol extract of *Azadirachta indica* and *Cymbopogon citratus*

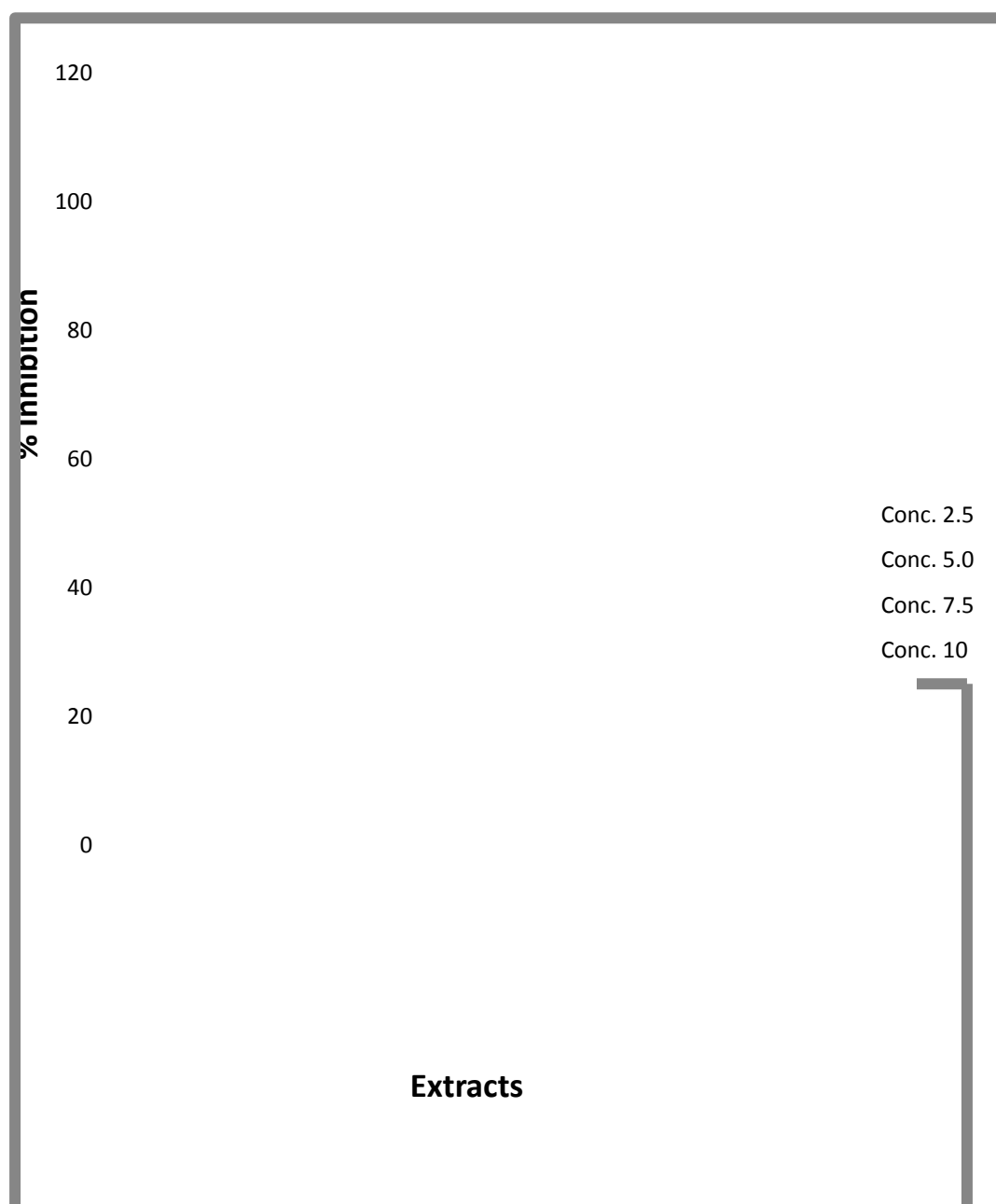
For the comparison between *V. amygdalina*, *C. papaya* and their combination on the inhibition of *Penicillium*, there was significant level of inhibition when the extracts were combined than when they were used individually. For ethanol extract, the combination of *V. amygdalina* and *C. citratus* gave an inhibition zone of 100% across all the concentrations; these were significantly ( $P < 0.05$ ) higher than values obtained from the individual plant extracts. The highest inhibitory value recorded from ethanol extract of *V. amygdalina* was 45% obtained at 7.5% concentration while ethanol extract of *C. papaya* showed 10% inhibition at 7.5% and 10% concentrations respectively. For aqueous extract, the combined plant extracts significantly ( $P < 0.05$ ) inhibited the growth of the test organism with values of 99%, 98%, 70% and 56% at 10%, 7.5%, 5% and 2.5% concentrations respectively as against 28%, 27.5%, 30% and 18% obtained from *V. amygdalina* at 10%, 7.5%, 5% and 2.5% concentrations respectively. *C. papaya* at all concentrations does not inhibit the growth of *Penicillium* spp (Figure 16).



**Figure 16: Comparison between aqueous and ethanol extracts of *Vernonia amygdalina* and *Carica papaya* and their synergistic effects on the inhibition of *Penicillium* spp**

Key: **A-Vernonia** means aqueous extract of *Vernonia amygdalina*, **A-Carica** Means aqueous extract of *Carica papaya*, **A-Vernonia + A-Carica** means the mixture of aqueous extract of *Vernonia amygdalina* and *Carica papaya*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina*, **E-Carica** Means ethanol extract of *Carica papaya* **E-Vernonia+ E-Carica** means the mixture of ethanol extract of *Vernonia amygdalina* and *Carica papaya*

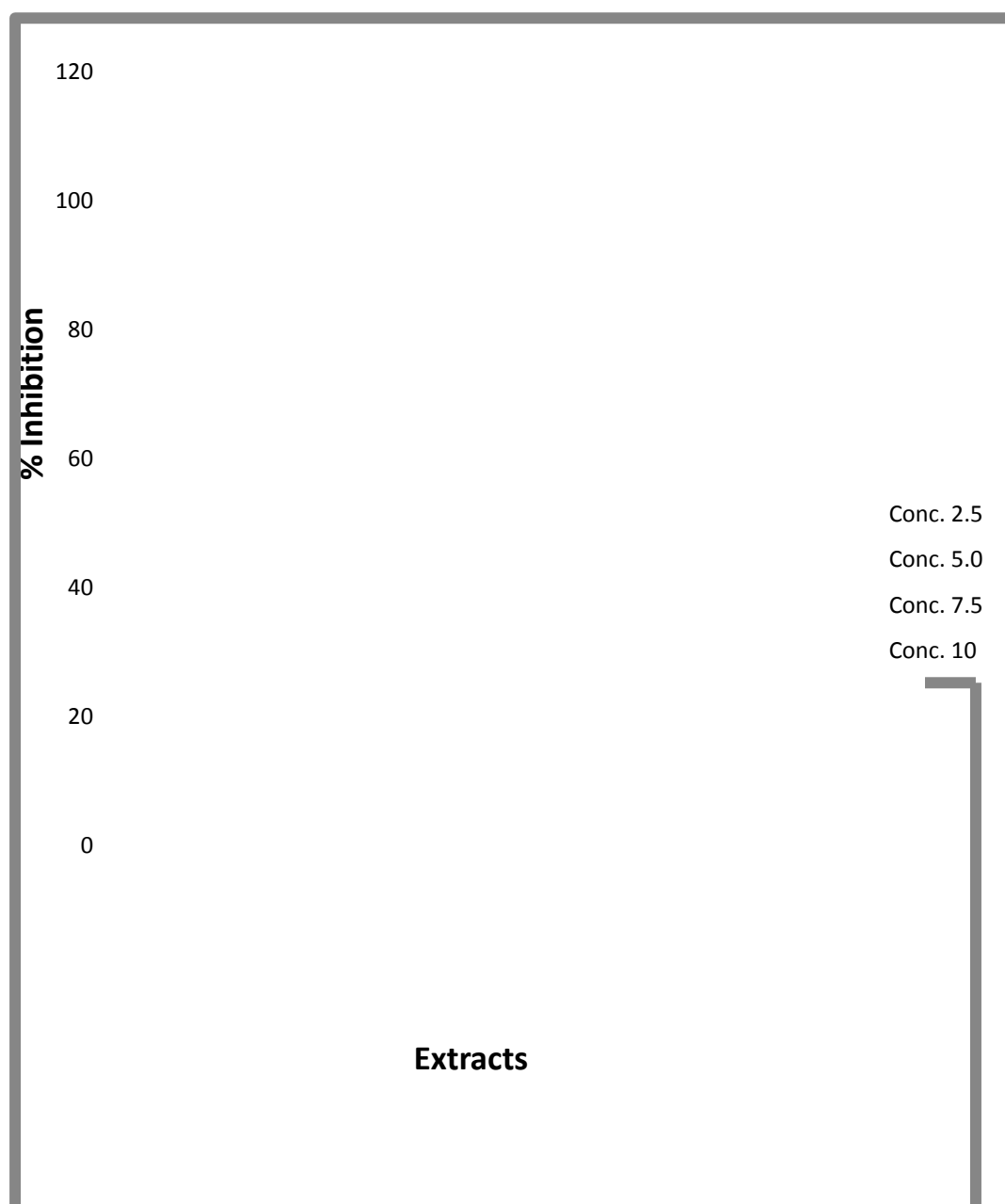
For the effect of *O. gratissimum*, *V. amygdalina* and their combination of the inhibition of *Penicillium* Spp, the combination of the extracts showed high synergistic effect against the test organism. With respect to ethanol, the combined plant extracts (*O. gratissimum* and *V. amygdalina*) proved to be more potent than the individual extracts by giving a value of 100% inhibition at 10% concentration and 90% at 7.5% and 5.0% concentrations each, while at 2.5% it gave an inhibition value of 84.5%. These values were significantly ( $P < 0.05$ ) higher than values from the individual plant extracts. *O. gratissimum* gave a value of 18% inhibition across all the concentrations, while *V. amygdalina* showed 44%, 38%, 37% and 18% inhibition at 7.5%, 10%, 5.0% and 2.5% concentrations respectively. With respect to aqueous, the combined plant extract especially at 7.5% and 10% concentration gave a value that was significantly ( $P < 0.05$ ) higher than values gotten from each of the individual extracts across all the concentrations. At 10% and 7.5%, the combined extract gave values of 98% and 96.5% respectively; these were significantly ( $P < 0.05$ ) higher than 38% and 36% inhibition observed from 10% and 7.5% aqueous extract of *V. amygdalina*. It is also significantly ( $P < 0.05$ ) higher than 18% and 15% inhibition values obtained from 10% and 7.5% aqueous extract of *O. gratissimum* (Figure 17).



**Figure 17: Comparison between aqueous and ethanol extracts of *Occimum gratissimum* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Penicillium* spp**

Key: **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Occimum + A-Vernonia** means the mixture of aqueous extract of *Occimum gratissimum* and *Vernonia amygdalina*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Occimum+ E-Vernonia** means the mixture of ethanol extract of *Occimum gratissimum* and *Vernonia amygdalina*

For the comparison between the effects of *A. indica*, *V. amygdalina* and their combination on the inhibition of *Penicillium* Spp, the effects of the combined extracts was more visible in ethanol extract than in aqueous extract. For ethanol, the values obtained from the combined plant extracts were 90%, 84%, 64% and 62% at 7.5%, 5.0%, 2.5% and 10% concentrations respectively. These were significantly higher than 46%, 38.8%, 37% and 17% obtained from ethanol extract of *V. amygdalina*. It was also significantly ( $P < 0.05$ ) higher than 46%, 44.5%, 30% and 18% obtained from ethanol extract of *V. amygdalina* at 7.5%, 10%, 5% and 2.5% concentrations respectively. For aqueous extract, the combined extract gave a relatively higher inhibition zone with values of 59%, 50%, 29% and 22% at 10%, 7.5%, 5.0% and 2.5% concentrations respectively. These were significantly ( $P < 0.05$ ) higher than values obtained from each of the individual extracts across all the concentrations (Figure 18).

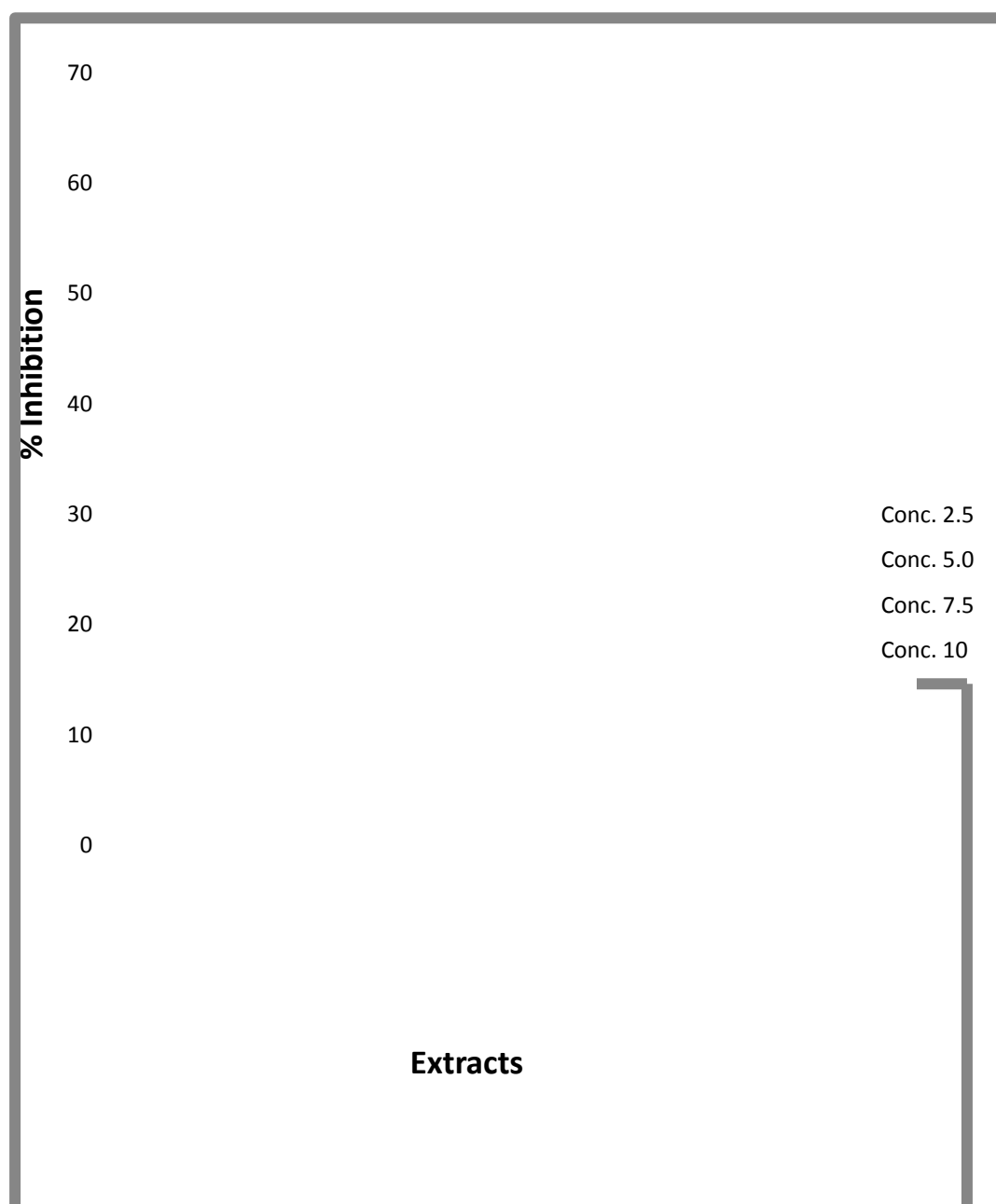


**Figure 18: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Penicillium spp***

**Key:** **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Azadi + A-Vernonia** means the mixture of aqueous extract of *Azadirachta indica* and *Vernonia amygdalina*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Azadi+ E-Vernonia** means the mixture of ethanol extract of *Azadirachta indica* and *Vernonia amygdalina*

For the effect of *C. citratus*, *O. gratissimum* and their combination on the inhibition of *Penicillium* spp, synergism only occurred in ethanol extracts. The combined ethanol extract of *C. sinensis* and *O. gratissimum* proved to be more effective than the individual extracts in the control of *Penicillium* spp. At 10% concentration a a very high inhibition zone was observed from the combined extracts with a value of 58%, this was significantly ( $P<0.05$ ) higher than 17% recorded from *O. gratissimum* and 18% recorded from *C. sinensis*. There was no synergism in the aqueous extract as the values obtained from the individual plant extracts and the combined plant extracts were not significantly ( $P<0.05$ ) different from each other (Figure 19).

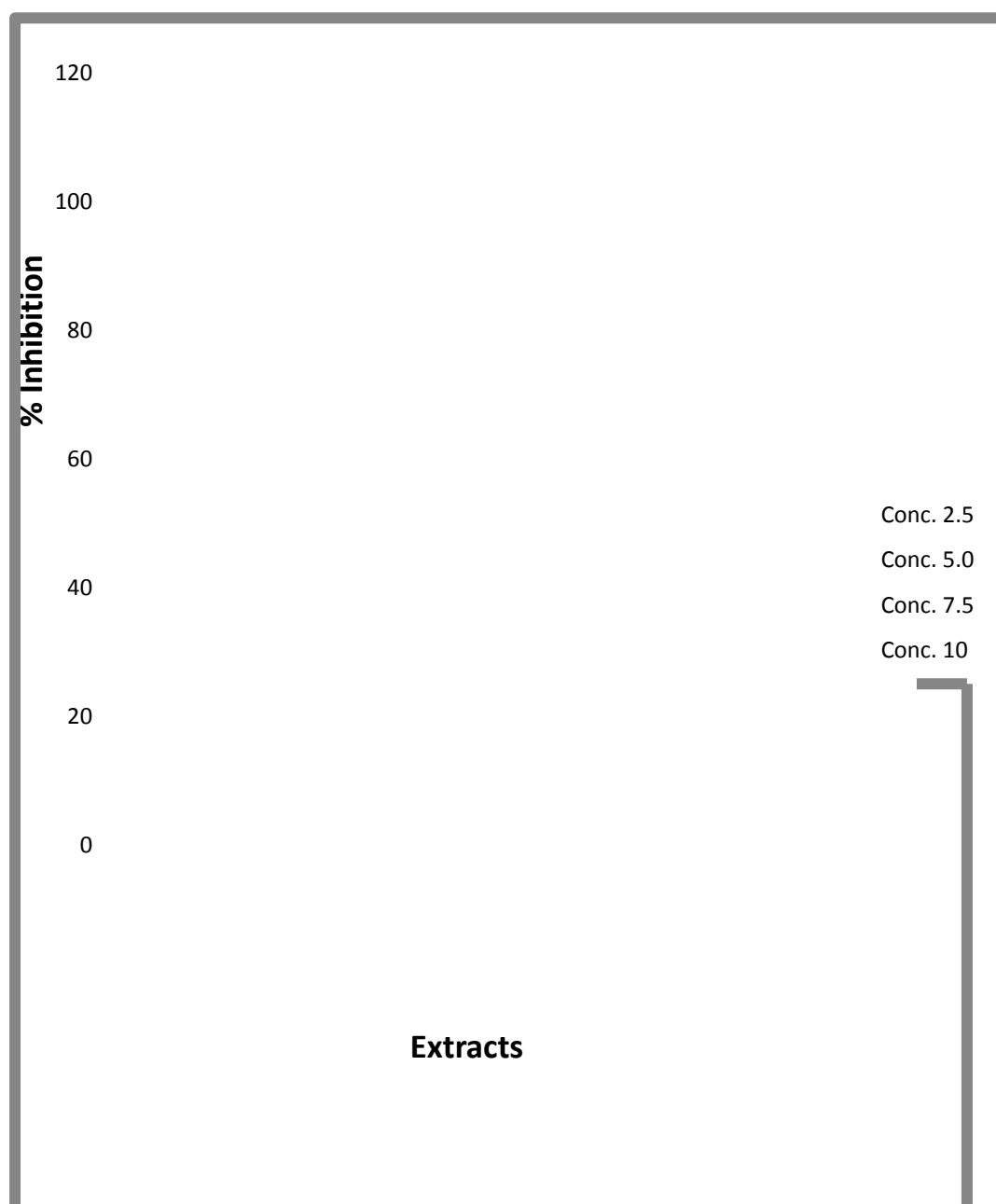




**Figure 19: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Occimum gratissimum* and their synergistic effects on the inhibition of *Penicillium spp***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-citrus +A-Occimum** means the mixture of aqueous extract of *Citrus sinensis* and *Occimum grassimum* , **E-Citrus** means ethanol extract of *Citrus sinensis*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Citrus + E-Occimum** means the mixture of ethanol extract of *Citrus sinensis* and *Occimum gratissimum*.

With respect to the effects of *C. papaya*, *C. citratus* and their combination on the inhibition of *Penicillium* Spp. The combined extracts show more inhibitory effects than their individual effects. For ethanol extract, a very high inhibition zone with value of 98% was obtained from the combined plant extracts at 7.5% and 10% concentrations while 5.0% and 2.5% concentrations gave inhibition values of 97% and 38% respectively. These values were significantly ( $P<0.05$ ) higher than 21% and 20% inhibition values obtained from ethanol extract of *C. citratus* at 10% and 7.5% concentrations respectively; ethanol extract of *C. papaya* also gave values of 10% inhibition at 7.5% and 10% concentrations each these were significantly ( $P<0.05$ ) lower than the values obtained from the combined plant extract. For aqueous extract, the combined plant extracts showed synergism by giving inhibition percentages of 40%, 36%, 33% and 30% at 10%, 5.0%, 7.5% and 2.5% concentrations respectively, this is against the lower values of 20%, 17%, 12% and 8% recorded from aqueous extract of *C. citratus* at 2.5%, 10%, 7.5% and 5.0% concentrations respectively. Aqueous extract of *C. papaya* does not inhibit the growth of *Penicillium* Spp at all the concentrations (Figure 20).



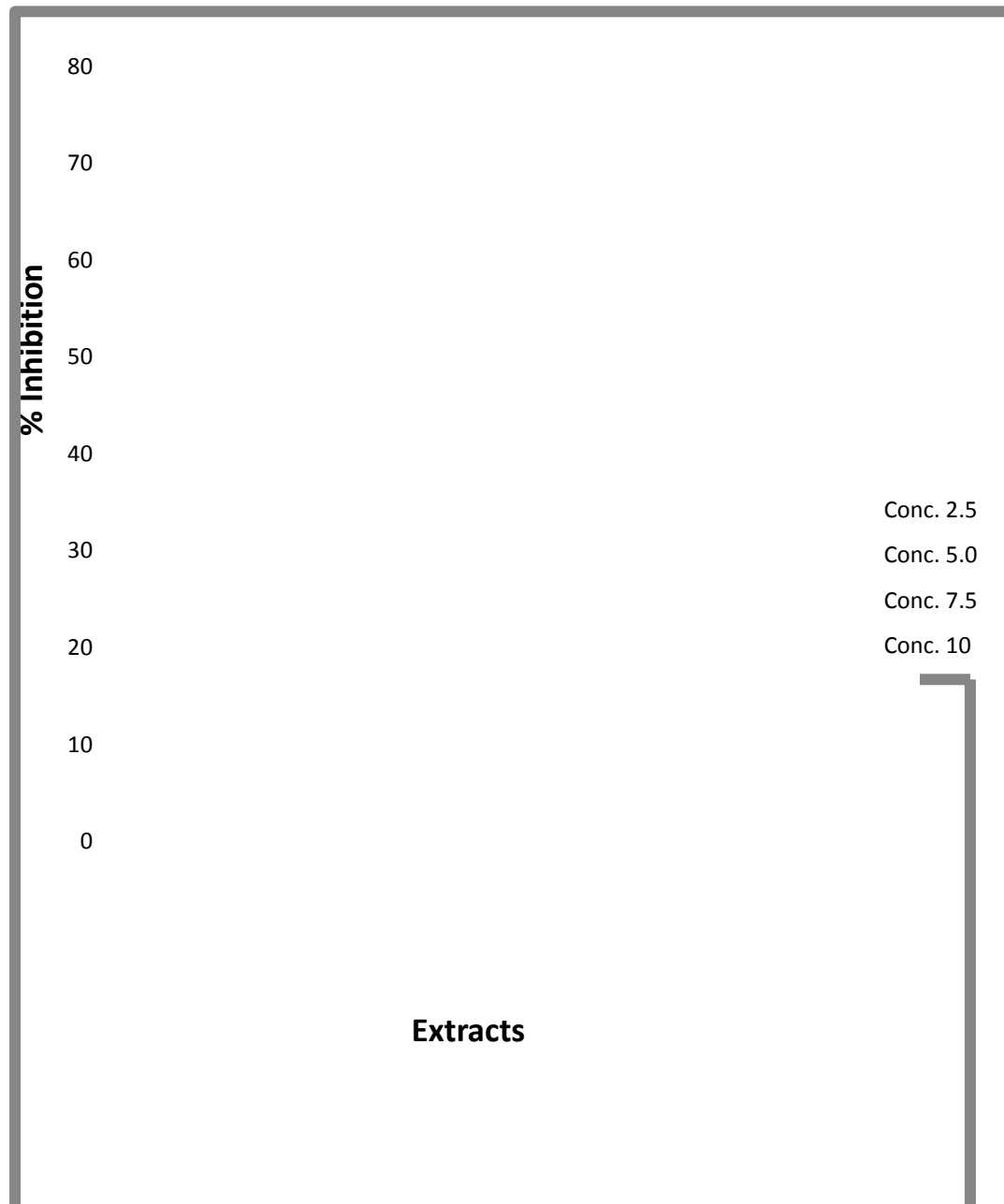
**Figure 20: Comparison between aqueous and ethanol extracts of *Carica papaya* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Penicillium spp***

Key: **A-Carica** means aqueous extract of *Carica papaya*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Carica + A-Cymbo** means the mixture of aqueous extract of *Carica papaya* and *Cymbopogon citratus*, **E-Carica** means ethanol extract of *Carica papaya*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus* **E-Carica+ E-Cymbo** means the mixture of ethanol extract of *Carica papaya* and *Cymbopogon citratus*

#### 4.6.3. *Rhizopus stolonifer*

Generally, the comparison between the effects of plant extracts and their combination on the inhibition of *Rhizopus stolonifer* was dependent on the extraction medium, hence ethanol extracts showed high degree of synergism, while aqueous extract did not show any synergistic effect.

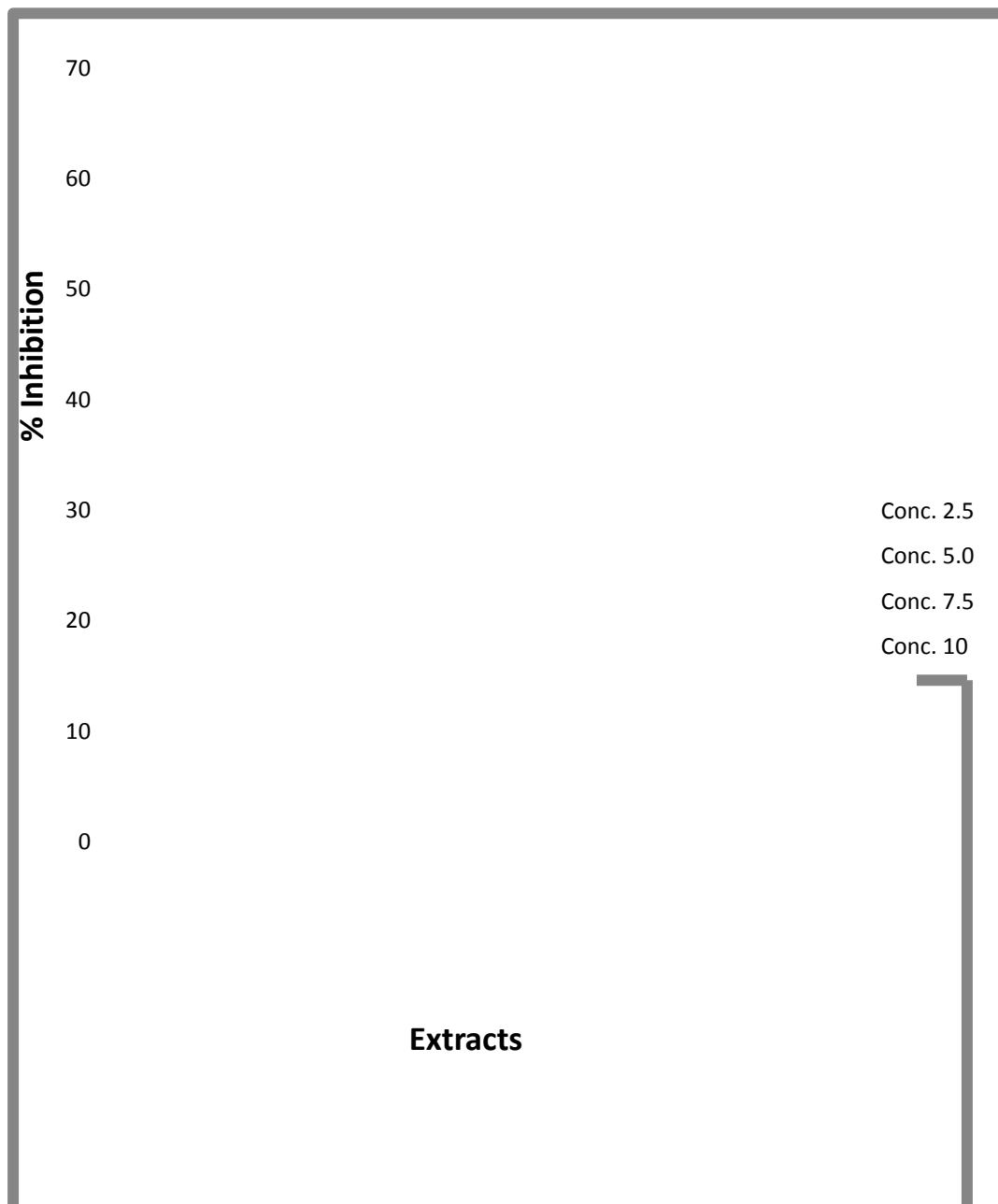
For the effects of *A. citratus*, *A. indica* and their combinations on the inhibition of *R. stolonifer*, the values recorded from the combined extracts were not significantly ( $P < 0.05$ ) different from the values obtained from the individual plant extracts; hence there was no synergistic effect except at 10% concentration. For ethanol medium, synergism only occurred at 10% concentration with a value of 63% this was significantly ( $P < 0.05$ ) higher than 46% and 18.5% inhibition obtained from *A. indica* and *C. sinensis* at 10% concentration each (Table 21).



**Figure 21: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Azadirachta indica* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Azadi** Means aqueous extract of *Azadirachta indica*, **A-Citrus + A-Azadi** means the mixture of aqueous extract of *Citrus sinensis* and *Azadirachta indica*, **E-Citrus** means ethanol extract of *Citrus sinensis* **E-Azadi** Means ethanol extract of *Azadirachta indica* **E-Citrus+ E-Azadi** means the mixture of ethanol extract of *Citrus sinensis* and *Azadirachta indica*

For the effects of *A.indica*, *C. citratus* and their combination on the inhibition of *R. stolonifer*, the combined extracts proved to be better than the individual extracts only in ethanol medium. The combined extracts gave a relatively higher inhibition zones with values of 56%, 51%, 38% and 22% at 10%, 7.5%, 5% and 2.5% concentrations respectively. These values were significantly ( $P<0.05$ ) higher than the highest value of 46% obtained from ethanol extract of *A. indica* and the highest value of 40% recorded from ethanol extract of *C. citratus* at 10% concentration. For aqueous, the combined plant extracts do not show any inhibition except slight inhibition at 10% concentration. The values obtained from the individual extracts were significantly ( $P<0.05$ ) higher than the values recorded when the extracts were mixed; hence they showed no synergistic effects on the inhibition of *R. stolonifer* (Figure 22).

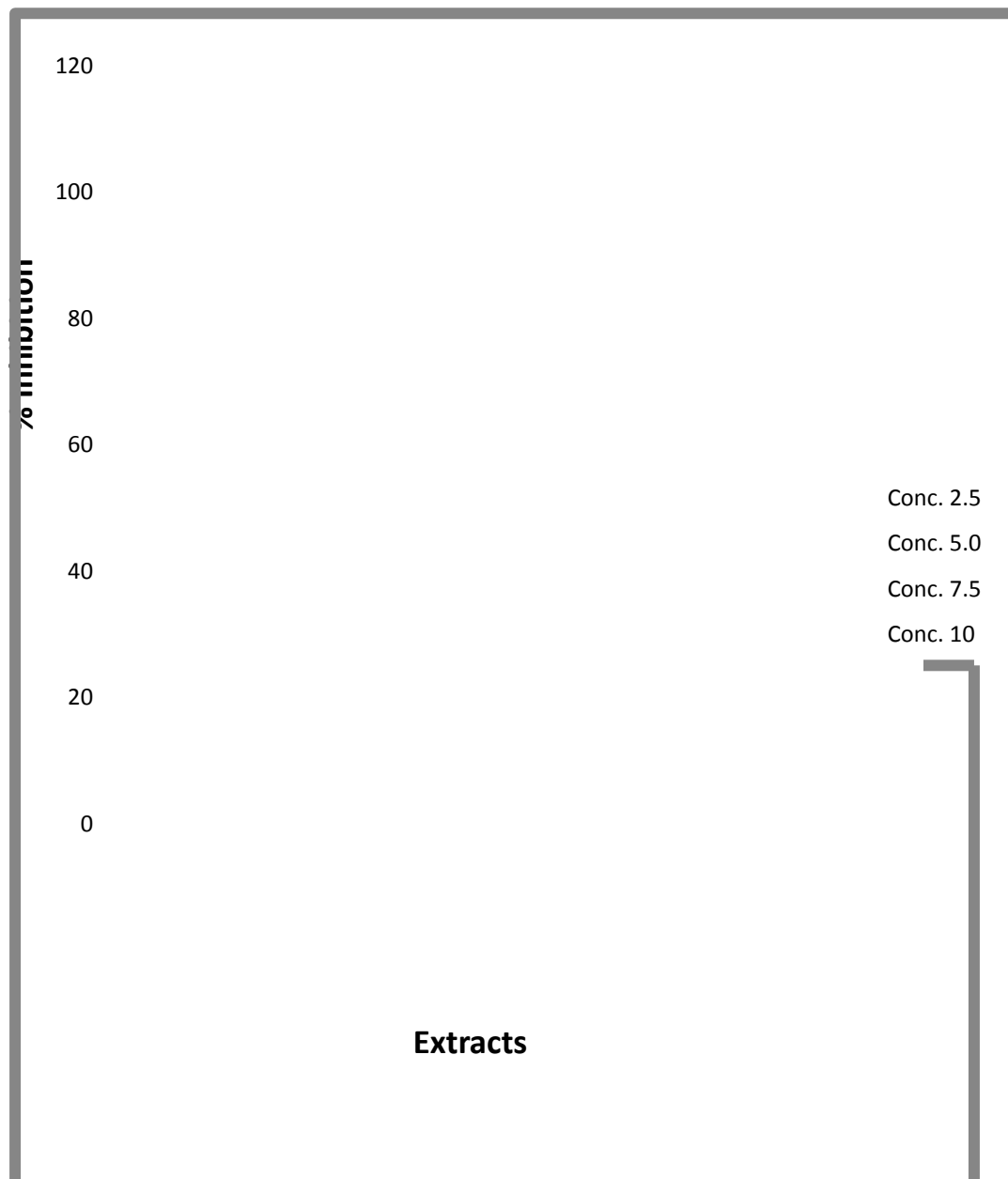


**Figure 22: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Azadi + A-Cymbo** means the mixture of aqueous extract of *Azadirachta indica* and *Cymbopogon citratus*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Cymbo** Means ethanol extract of *Cymbopogon citratus*, **E-Azadi + E-Cymbo** means the mixture of ethanol extract of *Azadirachta indica* and *Cymbopogon citratus*

With respect to the effect of *V. amygdalina*, *C. papaya* and their combination on the inhibition of *R. stolonifer*, their combination in aqueous across all the concentrations did not inhibit the growth of *R. stolonifer*, hence no synergism. With respect to ethanol, there was high level of synergism with the combined plant extracts giving an inhibition zone with values of 100% and 99% at 10% and 7.5% concentrations respectively and 41% and 40% inhibition at 5.0% and 2.5% respectively. These values were significantly ( $P < 0.05$ ) higher than values recorded from each of the individual extracts. The highest value of inhibition recorded from the individual extract was 40% from ethanol extract of *V. amygdalina* at 10% concentration, this was relatively lower than value obtained from the combination of the two extracts, hence there was synergism on their effects on *R. stolonifer* with respect to ethanol medium (Figure 23).

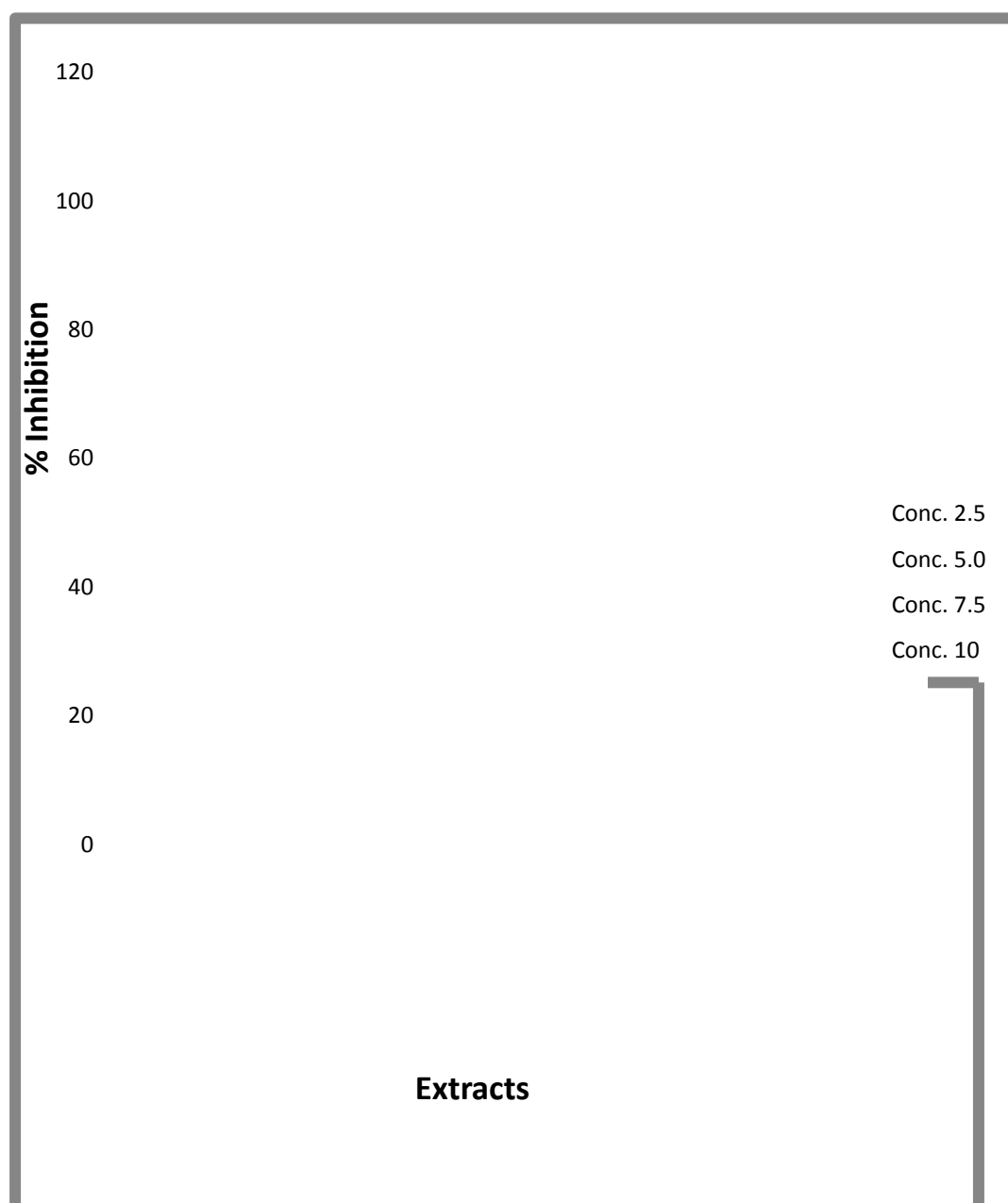




**Figure 23: Comparison between aqueous and ethanol extracts of *Vernonia amygdalina* and *Carica papaya* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

Key: **A-Vernonia** means aqueous extract of *Vernonia amygdalina*, **A-Carica** Means aqueous extract of *Carica papaya*, **A-Vernonia + A-Carica** means the mixture of aqueous extract of *Vernonia amygdalina* and *Carica papaya*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina*, **E-Carica** Means ethanol extract of *Carica papaya* **E-Vernonia+ E-Carica** means the mixture of ethanol extract of *Vernonia amygdalina* and *Carica papaya*

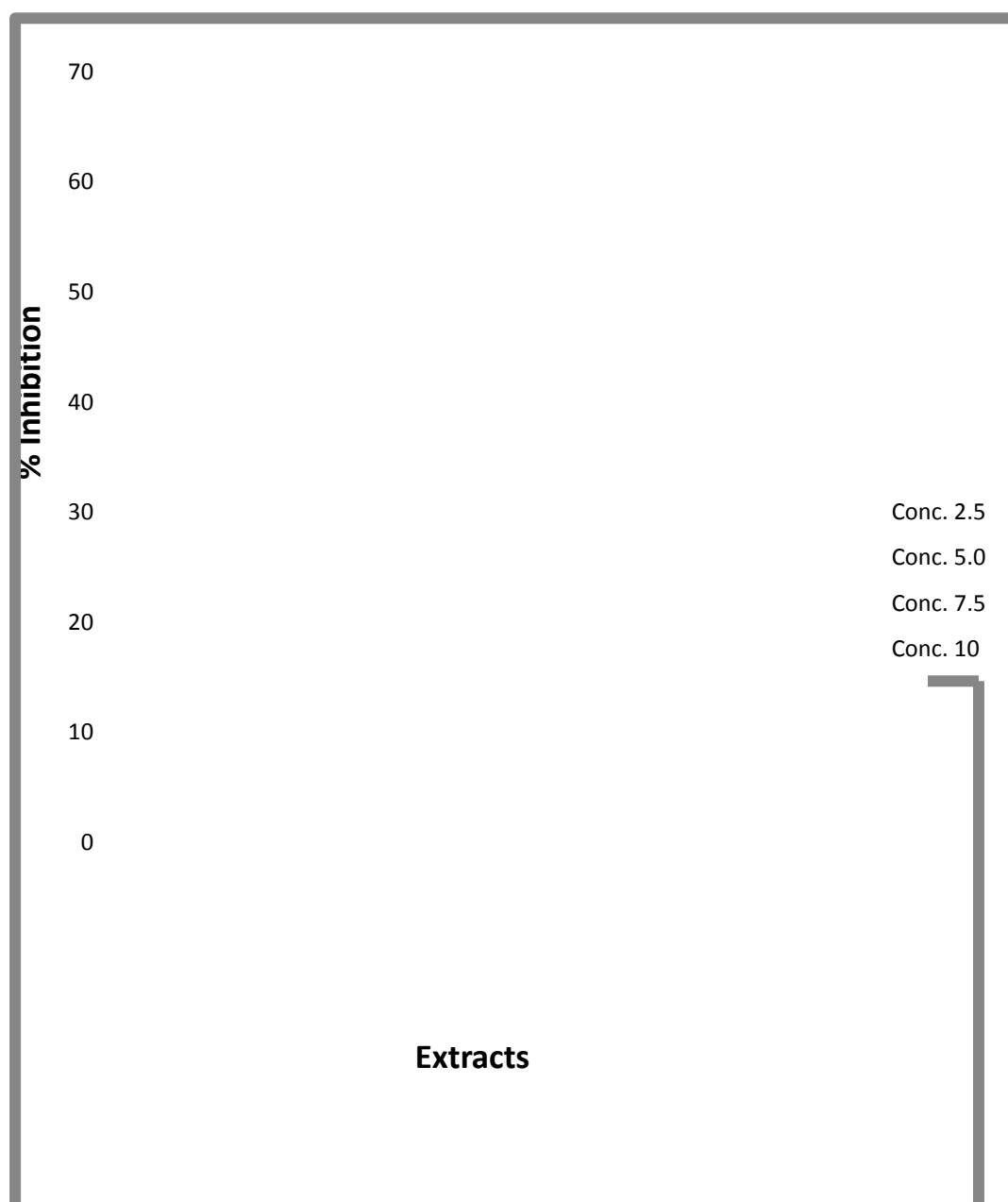
The effects of *O. gratissimum*, *V. amygdalina* and their combination on the inhibition of *R. stolonifer*, the effects of the combined extract varies with difference in extraction medium. Synergism only occurred in ethanol extract with values of 99%, 98%, 67% and 63% obtained at 10%, 7.5%, 5% and 2.5% concentrations respectively; these were significantly higher than the highest values of 40% obtained from the individual extracts. For aqueous, the combined plant extracts did not show any inhibition against *R. stolonifer*. The values obtained from the aqueous extract of the individual plant extracts were significantly ( $P < 0.05$ ) higher than the values obtained from the combined extracts, hence there was no synergism (Figure 24).



**Figure 24: Comparison between aqueous and ethanol extracts of *Occimum gratissimum* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

**Key:** **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Occimum + A-Vernonia** means the mixture of aqueous extract of *Occimum grassimum* and *Vernonia amygdalina*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Occimum+ E-Vernonia** means the mixture of ethanol extract of *Occimum gratissimum* and *Vernonia amygdalina*

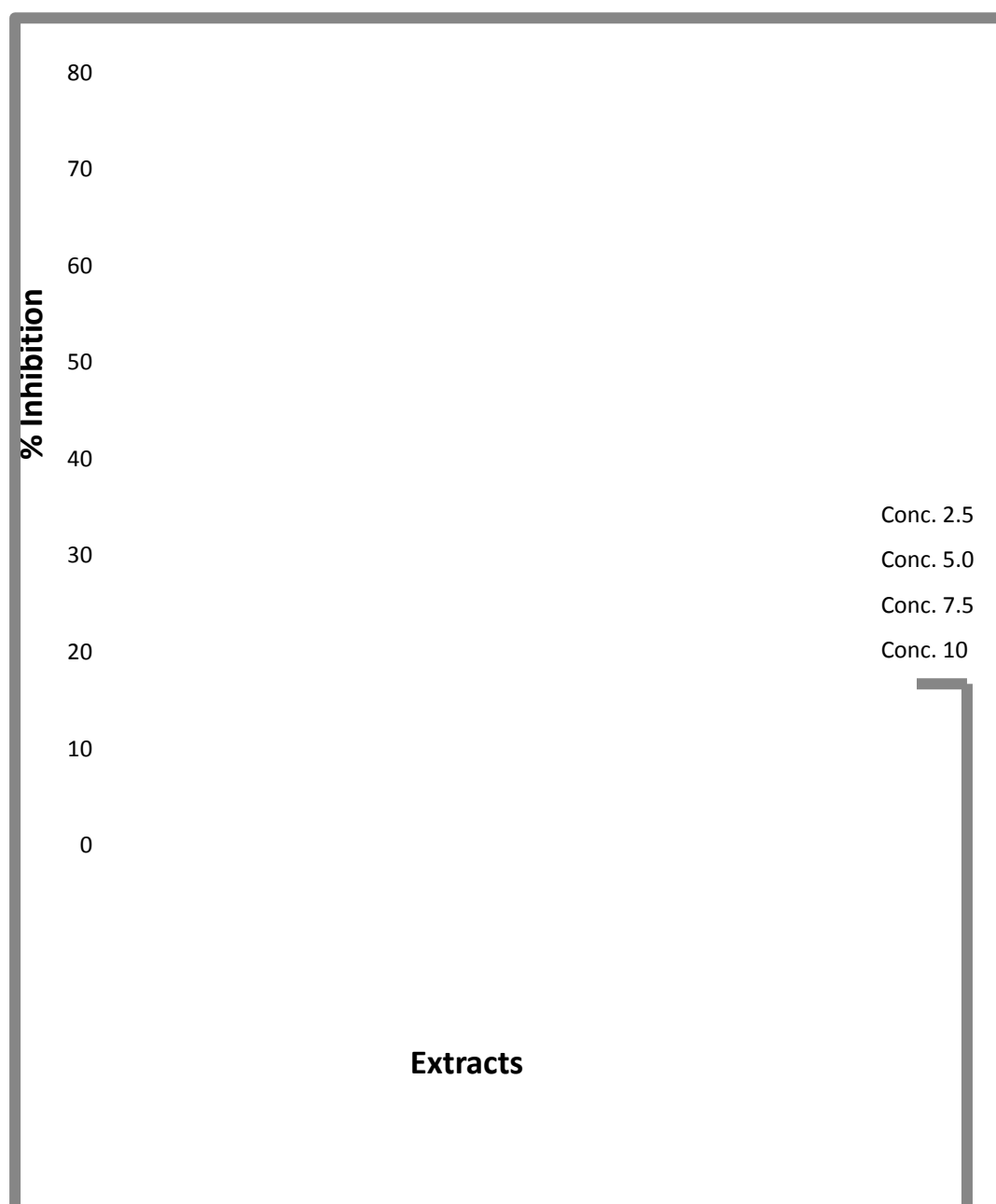
For *A. indica*, *V. amygdalina* and their combination on *R. stolonifer*, synergism only occurred in ethanol medium. For ethanol, the combined extract proved to be more potent in control the test fungi, the values obtained from the combined plant extracts were 61%, 44%, 25% and 20% and 10%, 7.5%, 5% and 2.5% concentrations respectively. These were significantly ( $P < 0.05$ ) higher than all the values obtained from the individual extracts. With respect to the individual extract, the highest zone of inhibition with value of 47% was obtained from ethanol extract of *A. indica* at 7.5% and 10% concentrations respectively, while the highest value of inhibition obtained from ethanol extract of *V. amygdalina* was 40% recorded at 10% concentration. For aqueous extract, the values obtained from the combined plant extracts were not significantly ( $P < 0.05$ ) higher than the values recorded from each individual plant extract, value of 33% obtained from aqueous extract of *A. indica* at 10% and 7.5% concentrations were significantly ( $P < 0.05$ ) higher than the values obtained from the combined extracts at all concentrations (Figure 25).



**Figure 25: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

**Key:** **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Azadi + A-Vernonia** means the mixture of aqueous extract of *Azadirachta indica* and *Vernonia amygdalina*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Azadi+ E-Vernonia** means the mixture of ethanol extract of *Azadirachta indica* and *Vernonia amygdalina*

For the effects of *C. citratus*, *O. gratissimum* and their combinations on the inhibition of *R. stolonifer*, the combined aqueous extract showed no inhibition on the test fungi hence there was no synergistic effect. With respect to ethanol extract, there was synergistic effect especially at 7.5%. The combined ethanol extract gave a high inhibition zone with values of 69% at 7.5% concentration and 35% inhibition at 2.5% and 5% concentrations each while a value of 22.5% was obtained from 10% concentration, these values were significantly ( $P < 0.05$ ) higher than values obtained from each of the individual extract across all the concentrations (Figure 26).

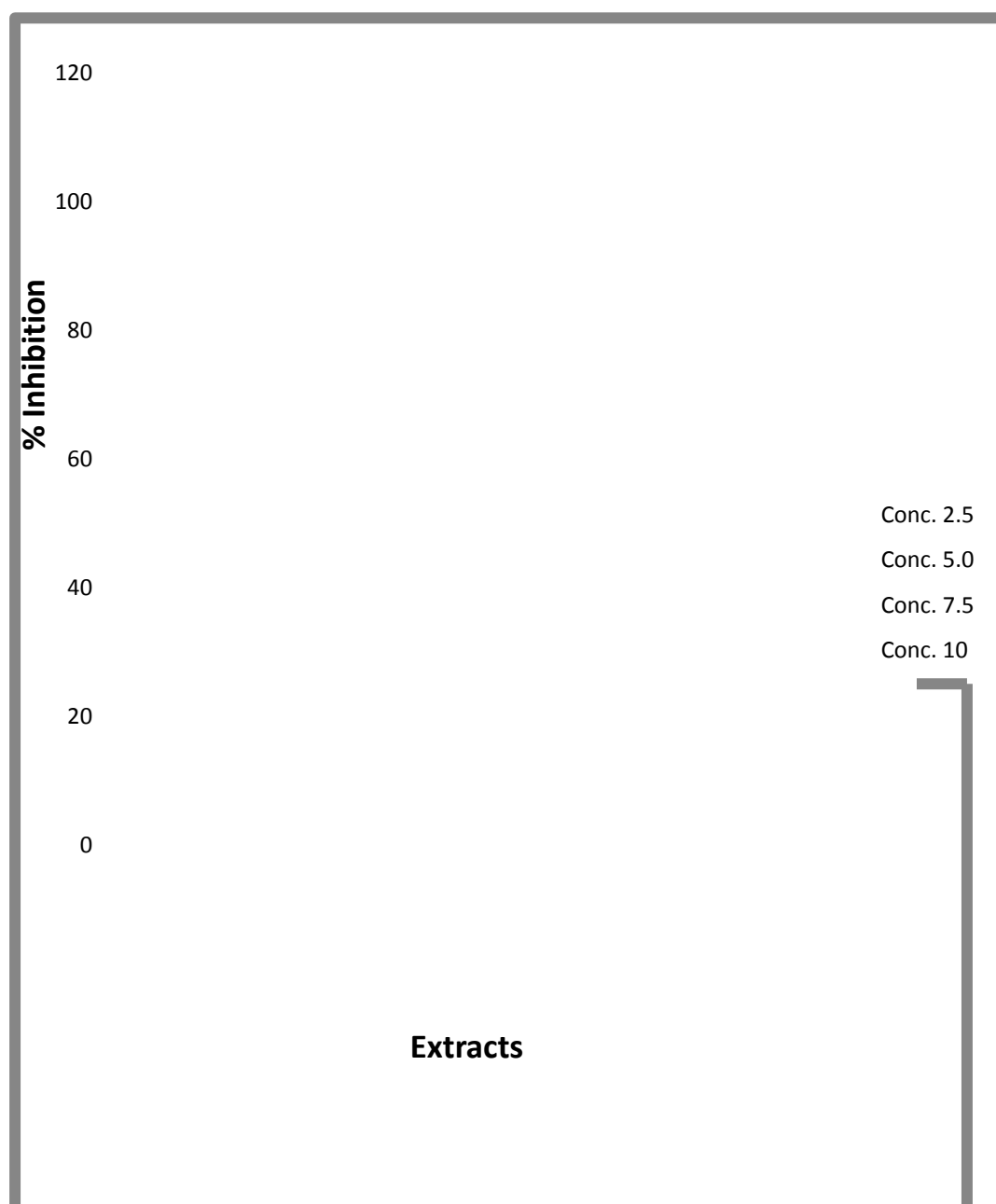


**Figure 26: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Occimum gratissimum* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-citrus +A-Occimum** means the mixture of aqueous extract of *Citrus sinensis* and *Occimum gratissimum*, **E-Citrus** means ethanol extract of *Citrus sinensis*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Citrus + E-Occimum** means the mixture of ethanol extract of *Citrus sinensis* and *Occimum gratissimum*.

The comparison between the effects of *C. papaya*, *C. citratus* and their combination on the inhibition of *R. stolonifer*, there was high degree of synergism across the extraction media. For ethanol, the combined plant extract at all the concentrations gave an inhibition zones that were significantly ( $P < 0.05$ ) higher than values obtained from each of the individual plant extracts. The values obtained from the combined extracts were 99% at 10%, 72% at 5% and 7.5% concentrations each, while 2.5% concentration gave an inhibition of 61%, these values were significantly ( $P < 0.05$ ) higher than the highest values of 40% and 30% recorded from ethanol extract of *C. citratus* and ethanol extract of *C. papaya* at 10% concentrations each. With respect to aqueous extract, the combined plant extracts also proved to be more potent than each of the individual extracts, the values of 42%, 41% and 37% obtained from the combined aqueous extract was significantly higher than the highest value of 23% obtained from the individual extract (Figure 27).



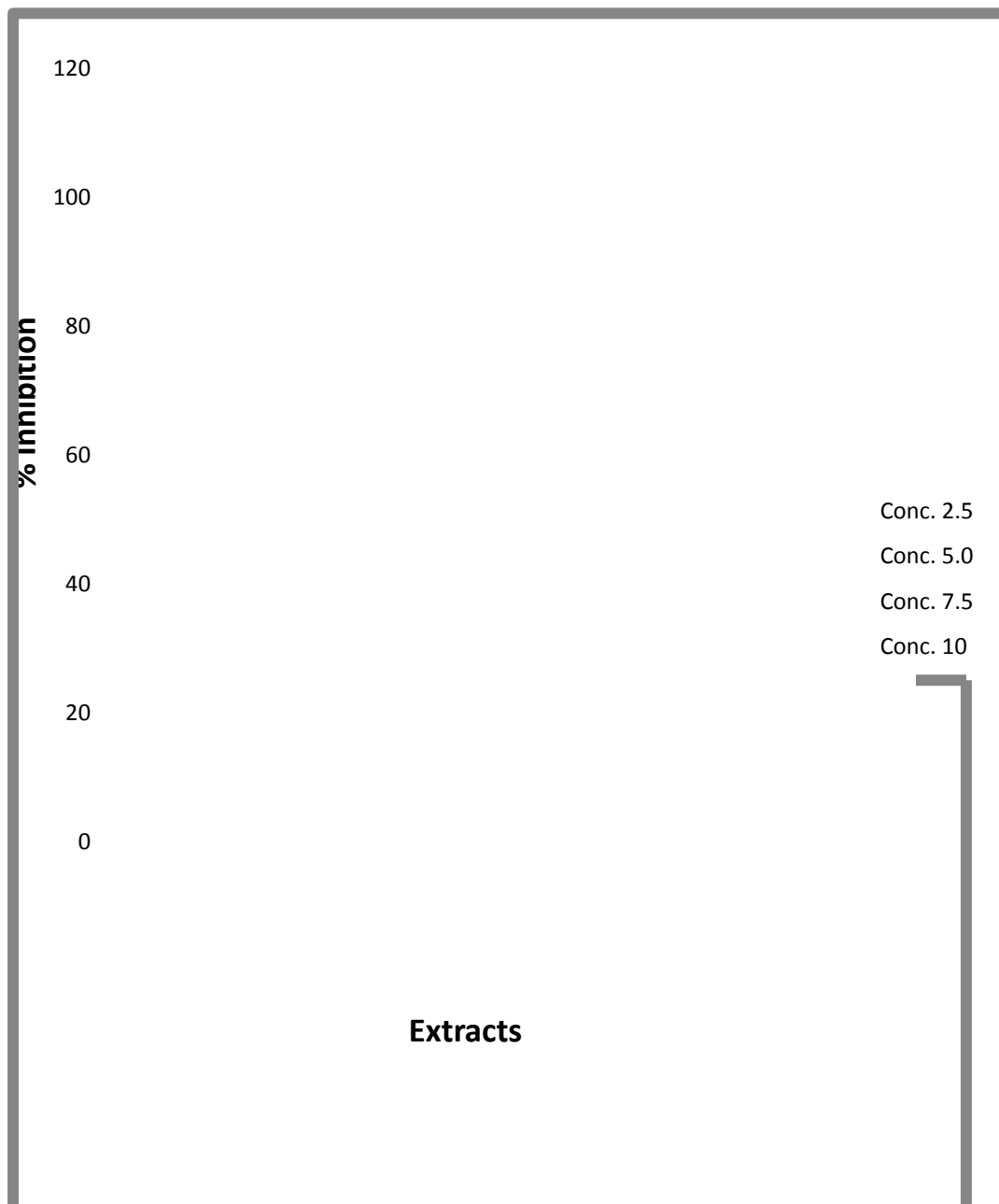


**Figure 27: Comparison between aqueous and ethanol extracts of *Carica papaya* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Rhizopus stolonifer***

Key: **A-Carica** means aqueous extract of *Carica papaya*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Carica + A-Cymbo** means the mixture of aqueous extract of *Carica papaya* and *Cymbopogon citratus*, **E-Carica** means ethanol extract of *Carica papaya*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus* **E-Carica+ E-Cymbo** means the mixture of ethanol extract of *Carica papaya* and *Cymbopogon citratus*

#### **4.6.4. *Botryodiplodia theobromae***

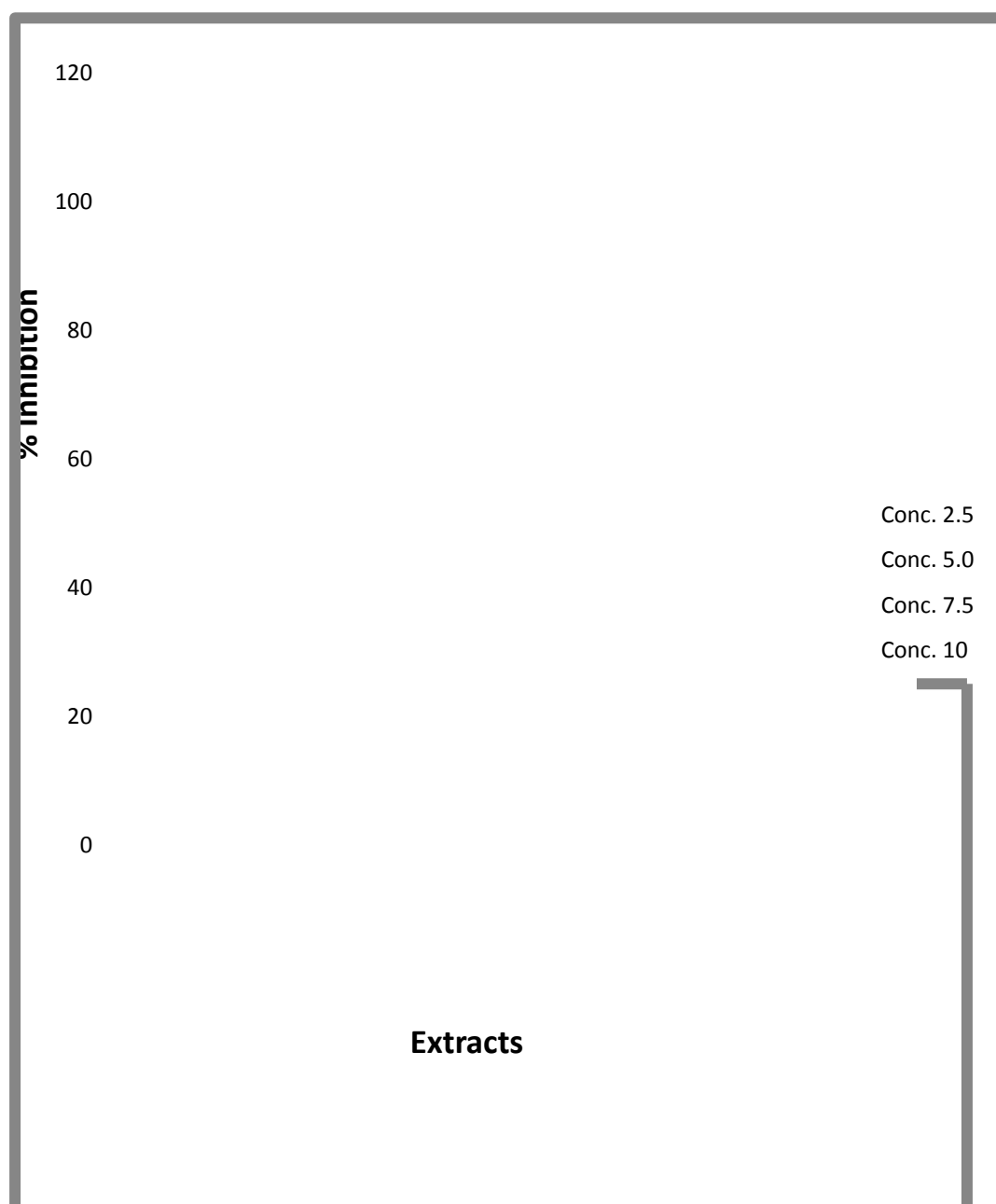
For the comparison between the effects of *C. citratus*, *A.indica* and their combination on the inhibition of *Botryodiplodia theobromae*, the combined plant extract in both ethanol and aqueous showed positive synergistic effect at 7.5% and 10% concentrations. With respect to ethanol, the combined extract gave the highest inhibition of 98% at 7.5% and 10% concentrations each, these values were significantly ( $P<0.05$ ) higher than the highest value of 49% obtained from *A. indica* at 7.5 and 10% concentrations and 20% inhibition obtained from *C. citratus* at all concentrations except at 2.5% concentration. For aqueous extract, the combined extracts at 10% and 7.5% proved to be more potent than the individual extracts (Figure 28).



**Figure 28: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Azadirachta indica* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Azadi** Means aqueous extract of *Azadirachta indica*, **A-Citrus + A-Azadi** means the mixture of aqueous extract of *Citrus sinensis* and *Azadirachta indica*, **E-Citrus** means ethanol extract of *Citrus sinensis* **E-Azadi** Means ethanol extract of *Azadirachta indica* **E-Citrus+ E-Azadi** means the mixture of ethanol extract of *Citrus sinensis* and *Azadirachta indica*

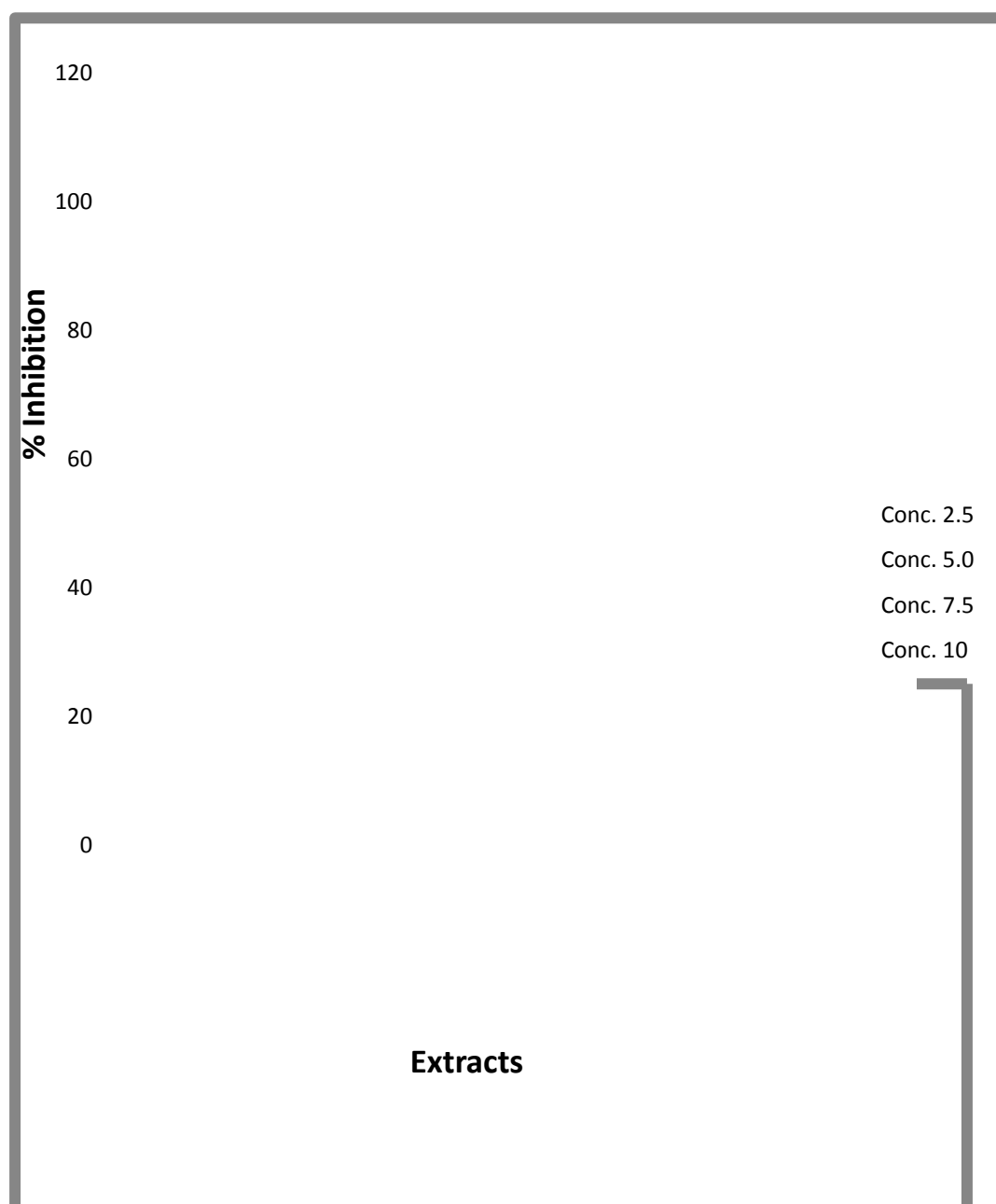
For the effects of *A. indica*, *C. citratus* and their combination on the inhibition of *Botryodiplodia theobromae*, there was synergistic effect on their inhibition on the test organisms. For ethanol, the combined extracted proved to be more efficacious in the inhibition of the organism with values of 100%, 98%, 85% and 66% at 10%, 7.5%, 5% and 2.5% concentrations respectively, these were significantly ( $P < 0.05$ ) higher than all the values obtained from the individual extracts at the corresponding concentrations. In aqueous, synergistic effect only occurred at 7.5% and 10% concentrations only with values of 38% and 69% respectively, the combined plant extract showed no inhibition at 2.5% and 5.0% concentrations, hence there was no synergism at these concentrations (Figure 29).



**Figure 29: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Azadi + A-Cymbo** means the mixture of aqueous extract of *Azadirachta indica* and *Cymbopogon citratus*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Cymbo** Means ethanol extract of *Cymbopogon citratus*, **E-Azadi + E-Cymbo** means the mixture of ethanol extract of *Azadirachta indica* and *Cymbopogon citratus*

Comparison between *V. amygdalina*, *C. papaya* and their combination on the inhibition of *Botryodiplodia theobromae* showed that synergism only occurred in ethanol extract. With respect to ethanol, the combined extract, a near complete at 10% concentration with value of 100%, while at 7.5% concentration the inhibition value of 98.5% was obtained, 5.0% and 2.5% concentrations recorded values of 71.5% and 67% respectively, these values were significantly ( $P<0.05$ ) higher than the highest value of 48% obtained from ethanol extract of *V. amygdalina* at 7.5% concentration. In aqueous, the combined extracts do not show any inhibition except very slight inhibition at 10% concentration, the values obtained from aqueous extract of *V. amygdalina* at 7.5% and 10% concentrations were significantly ( $P<0.05$ ) higher than the value obtained from the combined extracts, hence there was no synergism on the inhibition of *Botryodiplodia theobromae* (Figure 30).

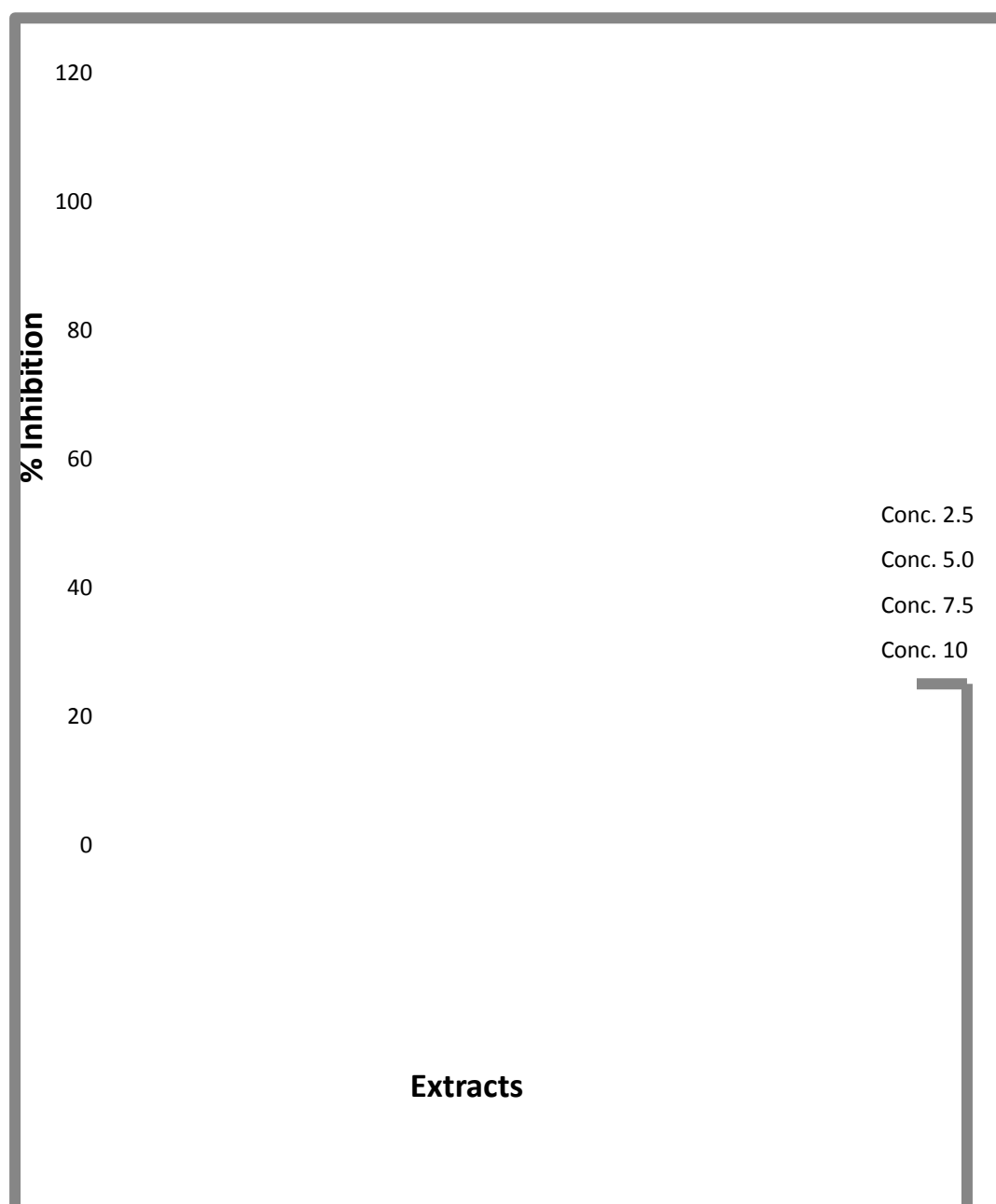


**Figure 30: Comparison between aqueous and ethanol extracts of *Vernonia amygdalina* and *Carica papaya* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

Key: **A-Vernonia** means aqueous extract of *Vernonia amygdalina*, **A-Carica** Means aqueous extract of *Carica papaya*, **A-Vernonia + A-Carica** means the mixture of aqueous extract of *Vernonia amygdalina* and *Carica papaya*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina*, **E-Carica** Means ethanol extract of *Carica papaya* **E-Vernonia+ E-Carica** means the mixture of ethanol extract of *Vernonia amygdalina* and *Carica papaya*

The comparative effects of *O. gratissimum*, *V. amygdalina* and their combination on the inhibition of *Botryodiplodia theobromae* showed that the effect of the combined extract was dependent on the solvent of extraction. In ethanol, the combined extract gave 100% inhibition across all the concentrations, this was significantly ( $P < 0.05$ ) higher than 46%, 40%, 27% and 20% inhibition obtained from ethanol extract of *V. amygdalina* at 7.5%, 10%, 5% and 2.5% respectively. Ethanol extract of *O. gratissimum* also gave relatively lower zones of inhibition with values of 28% at 7.5% concentration and 20% at 10% and 5% concentrations each. In aqueous, the combined extracts did not inhibit the growth of *Botryodiplodia theobromae*, hence aqueous extract showed no synergism (Figure 31).

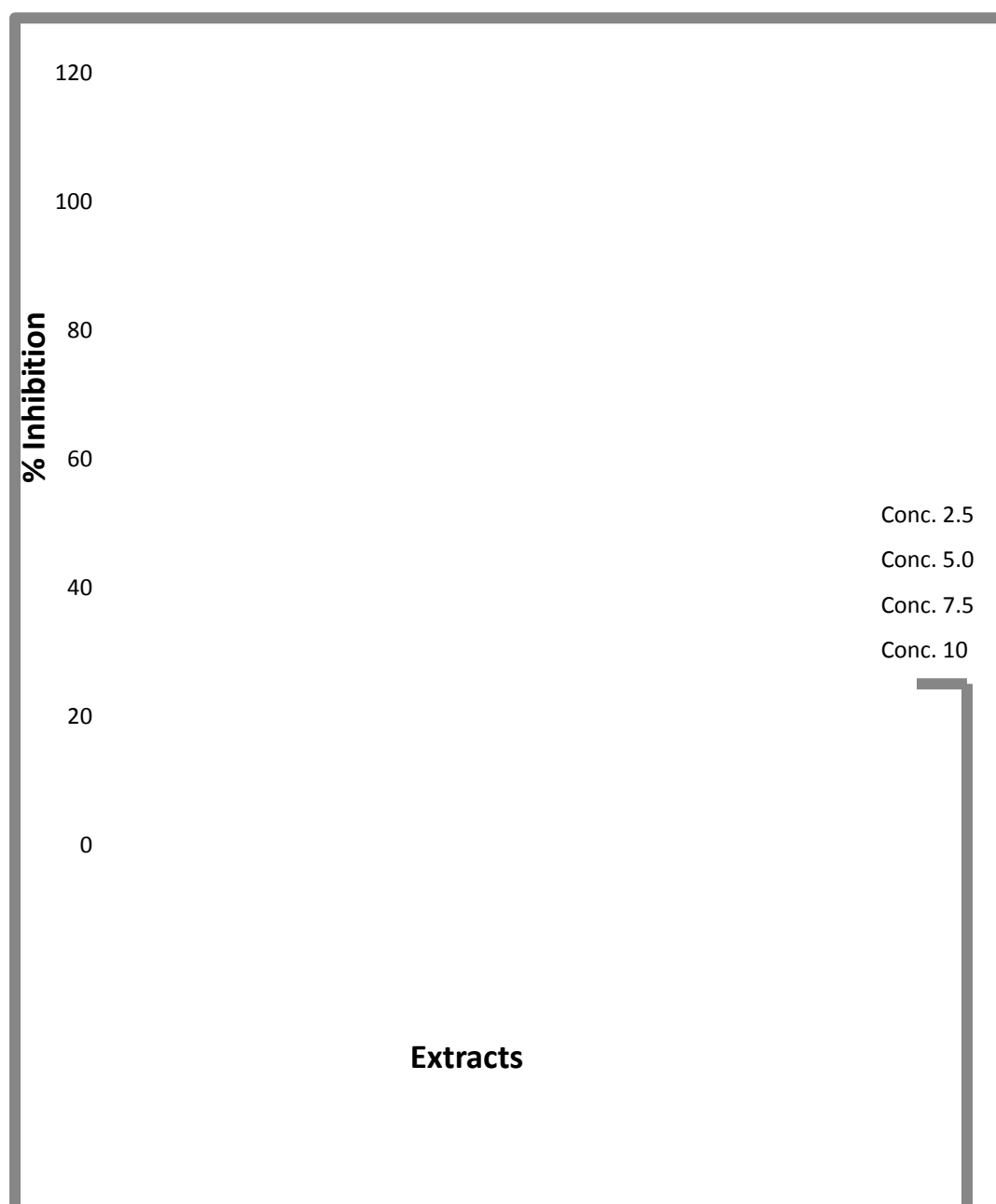




**Figure 31: Comparison between aqueous and ethanol extracts of *Occimum gratissimum* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

**Key:** **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Occimum + A-Vernonia** means the mixture of aqueous extract of *Occimum gratissimum* and *Vernonia amygdalina*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Occimum+ E-Vernonia** means the mixture of ethanol extract of *Occimum gratissimum* and *Vernonia amygdalina*

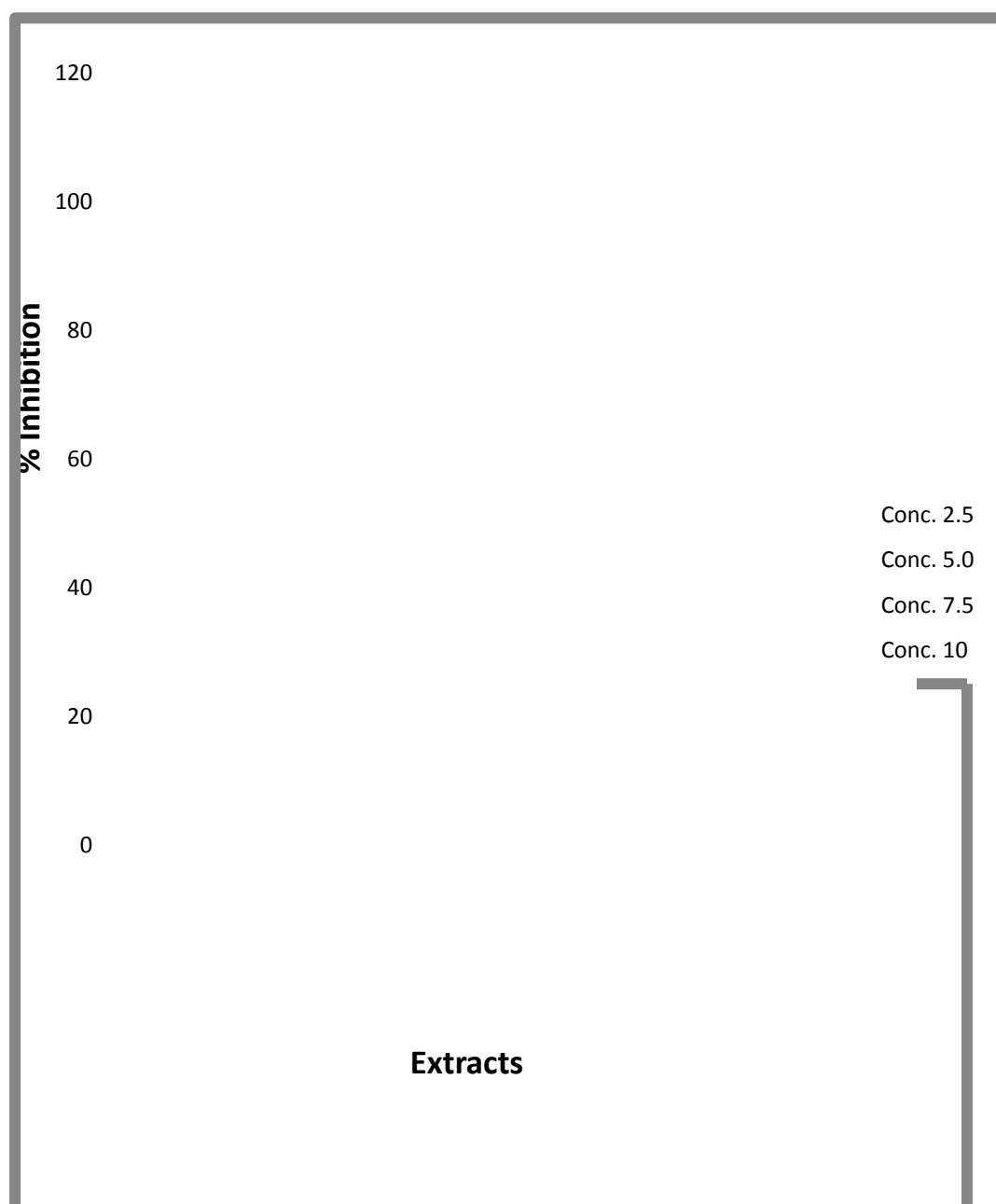
For *A. indica*, *V. amygdalina* and their combination on the inhibition of *Botryodiplodia theobromae*, synergism only occurred in aqueous extract. For aqueous extract, almost all concentrations of the combined extract gave a near complete inhibition with values ranging between 96% and 97%, these were significantly ( $P < 0.05$ ) higher than the highest value obtained from each of the individual extract. The values of 38%, 30%, 12% and 5% were obtained from aqueous extract of *V. amygdalina* at 7.5%, 10%, 5% and 2.5% concentrations respectively while 30% inhibition was obtained from aqueous extract of *A. indica* at 7.5% and 10% concentrations each. In ethanol, the values obtained from the combined plant extract was not significantly ( $P < 0.05$ ) different from values obtained from other interactions, hence there was no synergism with respect to ethanol extract (Figure 32).



**Figure 32: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

**Key:** **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Azadi + A-Vernonia** means the mixture of aqueous extract of *Azadirachta indica* and *Vernonia amygdalina*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Azadi+ E-Vernonia** means the mixture of ethanol extract of *Azadirachta indica* and *Vernonia amygdalina*

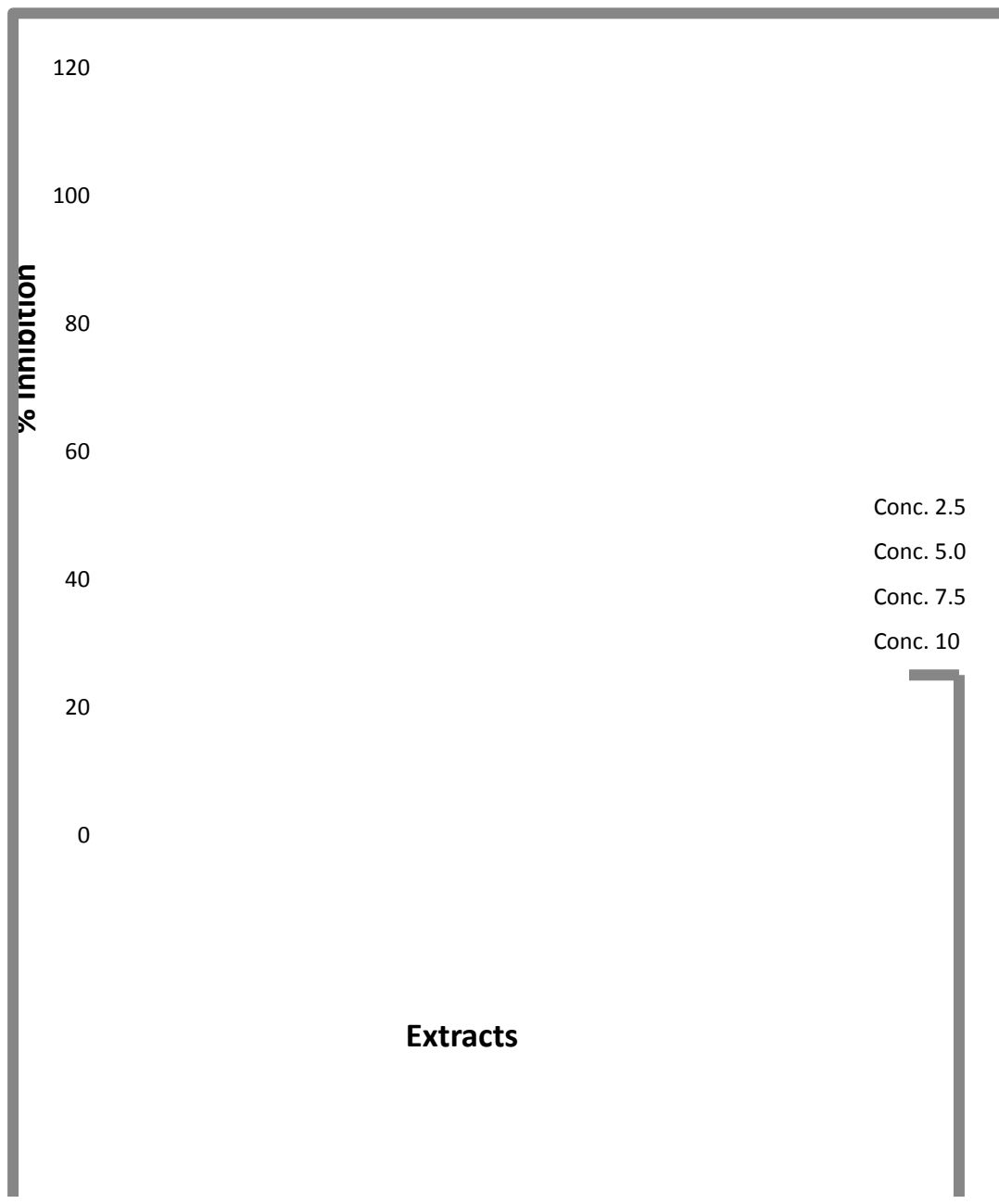
For the comparative effects of *C. sinensis*, *O. gratissimum* and their combination on the inhibition of *Botryodiplodia theobromae*, both aqueous and ethanol extracts exhibited very high degree of synergism. In ethanol, the combined extract gave a relatively high inhibition zone with values of 73.5%, 69%, 58% and 30% at 7.5%, 10%, 5% and 2.5% concentrations respectively, these values were significantly ( $P < 0.05$ ) higher than values gotten from each of the individual plant extracts, with respect to individual extract, the highest values obtained from ethanol extract of *O. gratissimum* was 27% observed at 7.5% concentration while ethanol extract of *C. sinensis* gave an inhibition value of 20% at all the concentrations except at 2.5% concentration. In aqueous, the combined extract had more inhibitory effect than the individual plant extracts. The combined extract gave a very high inhibition with values ranging from 90% and 95%. These were significantly ( $P < 0.05$ ) higher than the highest value of 20% obtained from the individual extracts (Figure 33).



**Figure 33: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Occimum gratissimum* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-citrus +A-Occimum** means the mixture of aqueous extract of *Citrus sinensis* and *Occimum grassimum* , **E-Citrus** means ethanol extract of *Citrus sinensis*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Citrus + E-Occimum** means the mixture of ethanol extract of *Citrus sinensis* and *Occimum gratissimum*.

The comparative effects of *C. papaya*, *C. citratus* and their combination on the inhibition *Botryodiplodia theobromae* depicted that synergism only occurred in ethanol extracts. In ethanol, the combined extract gave a very high inhibition values with values that are significantly higher than values recorded from each of the individual extract. The combined extract completely inhibited (100%) the growth of the test organism at all the concentrations except at 2.5% where it gave 98% inhibition, while the individual extract showed very slight inhibition with the highest value of 30% obtained from *C. citratus* at 7.5% and 10% concentrations each. In aqueous, the combined extracts showed no inhibition at all the concentration except at 2.5%, hence there was no synergism at all the concentrations except at 10% concentration with a value of 5% (Figure 34).



**Figure 34: Comparison between aqueous and ethanol extracts of *Carica papaya* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Botryodiplodia theobromae***

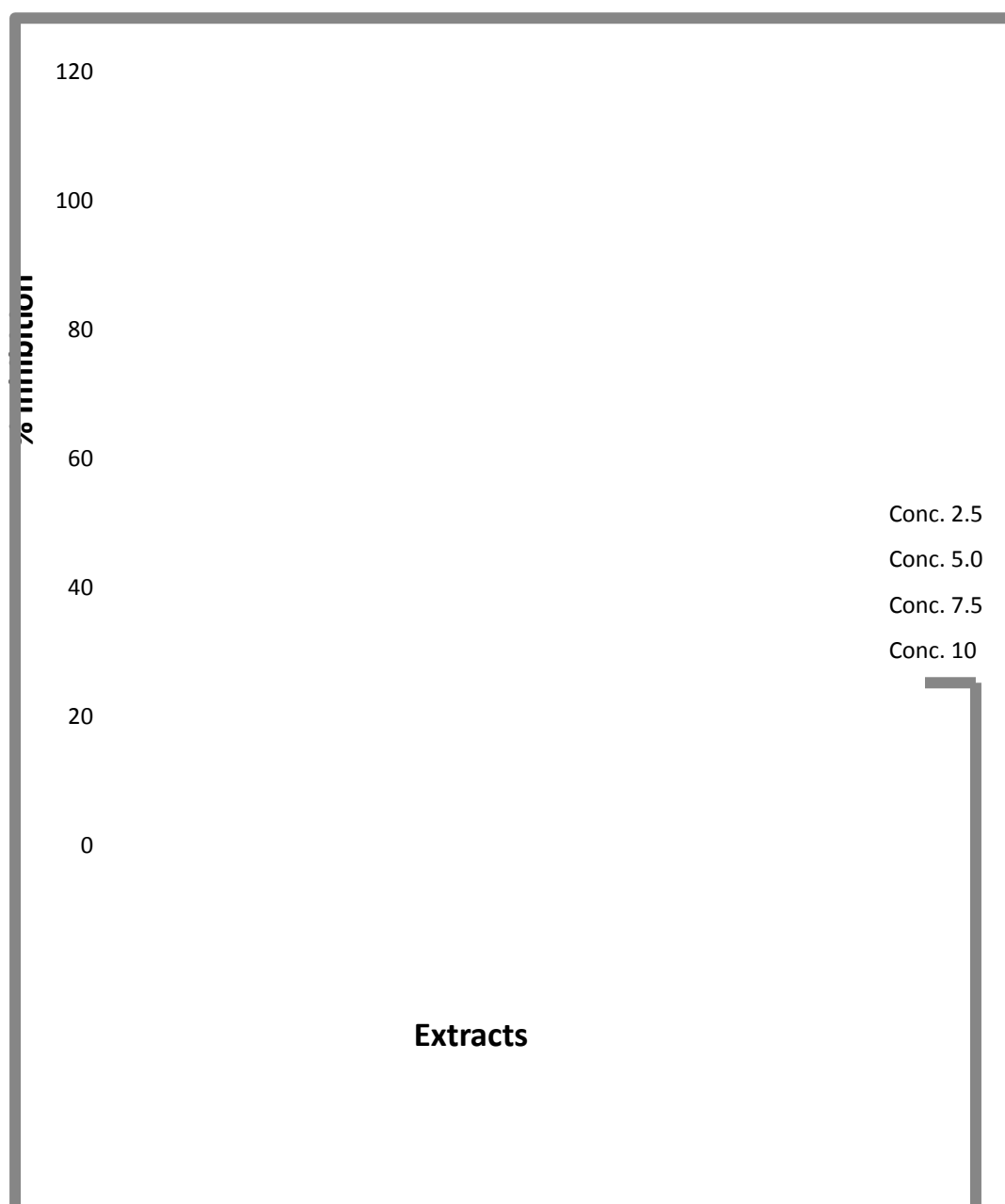
Key: **A-Carica** means aqueous extract of *Carica papaya*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Carica + A-Cymbo** means the mixture of aqueous extract of *Carica papaya* and *Cymbopogon citratus*, **E-Carica** means ethanol extract of *Carica papaya*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus* **E-Carica+ E-Cymbo** means the mixture of ethanol extract of *Carica papaya* and *Cymbopogon citratus*

#### **4.6.5. *Aspergillus flavus***

For the comparison between the effects of aqueous and ethanol extracts and their combinations on the inhibition of *A. niger*, synergism exists between the two extracts both in aqueous and in ethanol.

With respect to the effects of *C. citratus*, *A. indica* and their combination, the combined extracts proved to be more potent than the individual extracts on the inhibition of the test fungi. For ethanol, the combined extract completely inhibited the growth of the organism at 10% concentration, while it gave the value of 98% and 97% at 7.5% and 5% concentrations respectively. These were significantly ( $P < 0.05$ ) higher than the highest value of 50% obtained from the individual extracts. In aqueous, the combined extract gave 100% inhibition at 10% concentration, while it gave 98% inhibition at 7.5% concentration, it also gave the values of 70% and 67% at 5.0% and 2.5% concentrations respectively, these were significantly ( $P < 0.05$ ) higher than all the values obtained from the individual extracts (Figure 35).

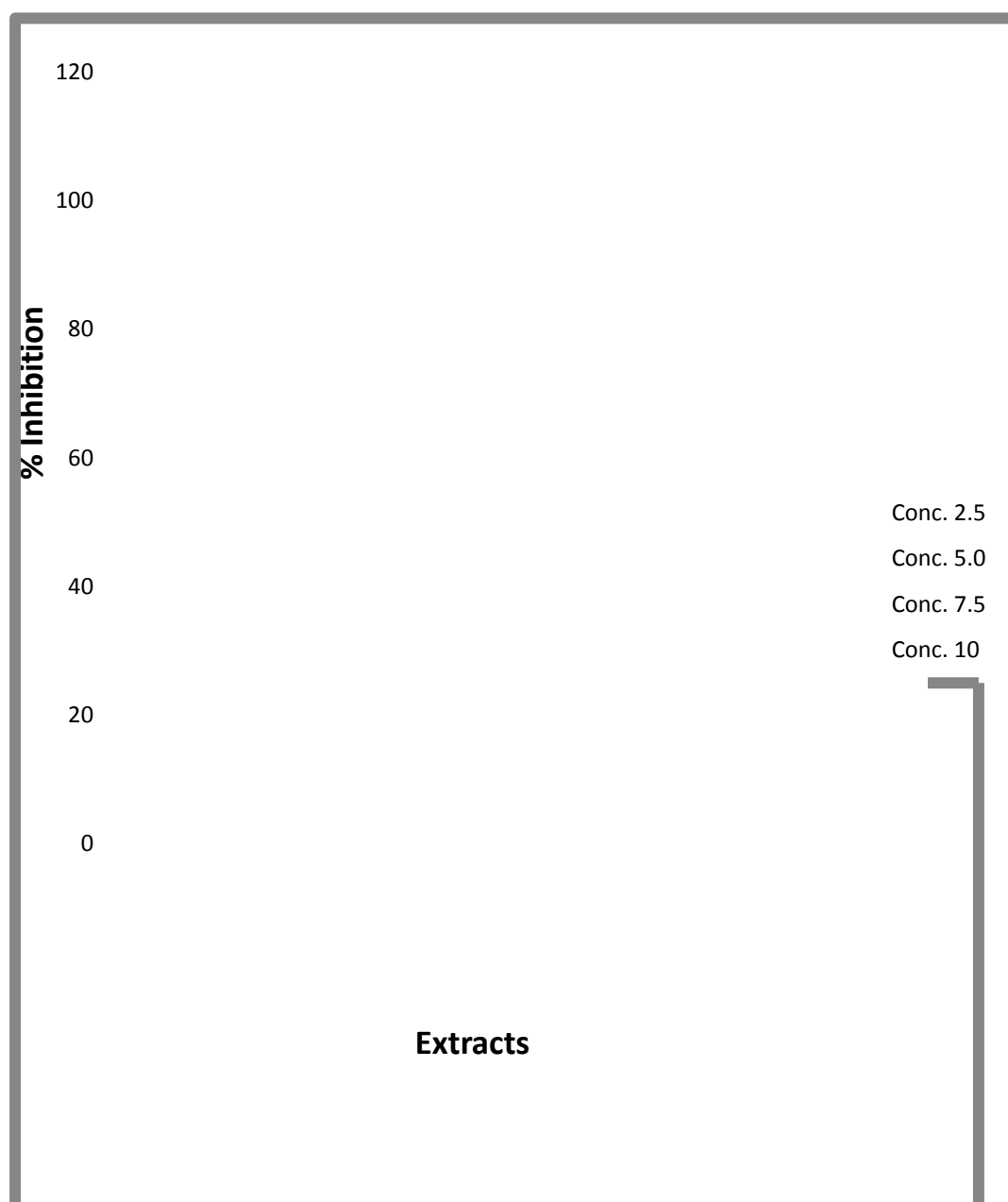




**Figure 35: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Azadirachta indica* and their synergistic effects on the inhibition of *Aspergillus flavus***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Azadi** Means aqueous extract of *Azadirachta indica*, **A-Citrus + A-Azadi** means the mixture of aqueous extract of *Citrus sinensis* and *Azadirachta indica*, **E-Citrus** means ethanol extract of *Citrus sinensis* **E-Azadi** Means ethanol extract of *Azadirachta indica* **E-Citrus+ E-Azadi** means the mixture of ethanol extract of *Citrus sinensis* and *Azadirachta indica*

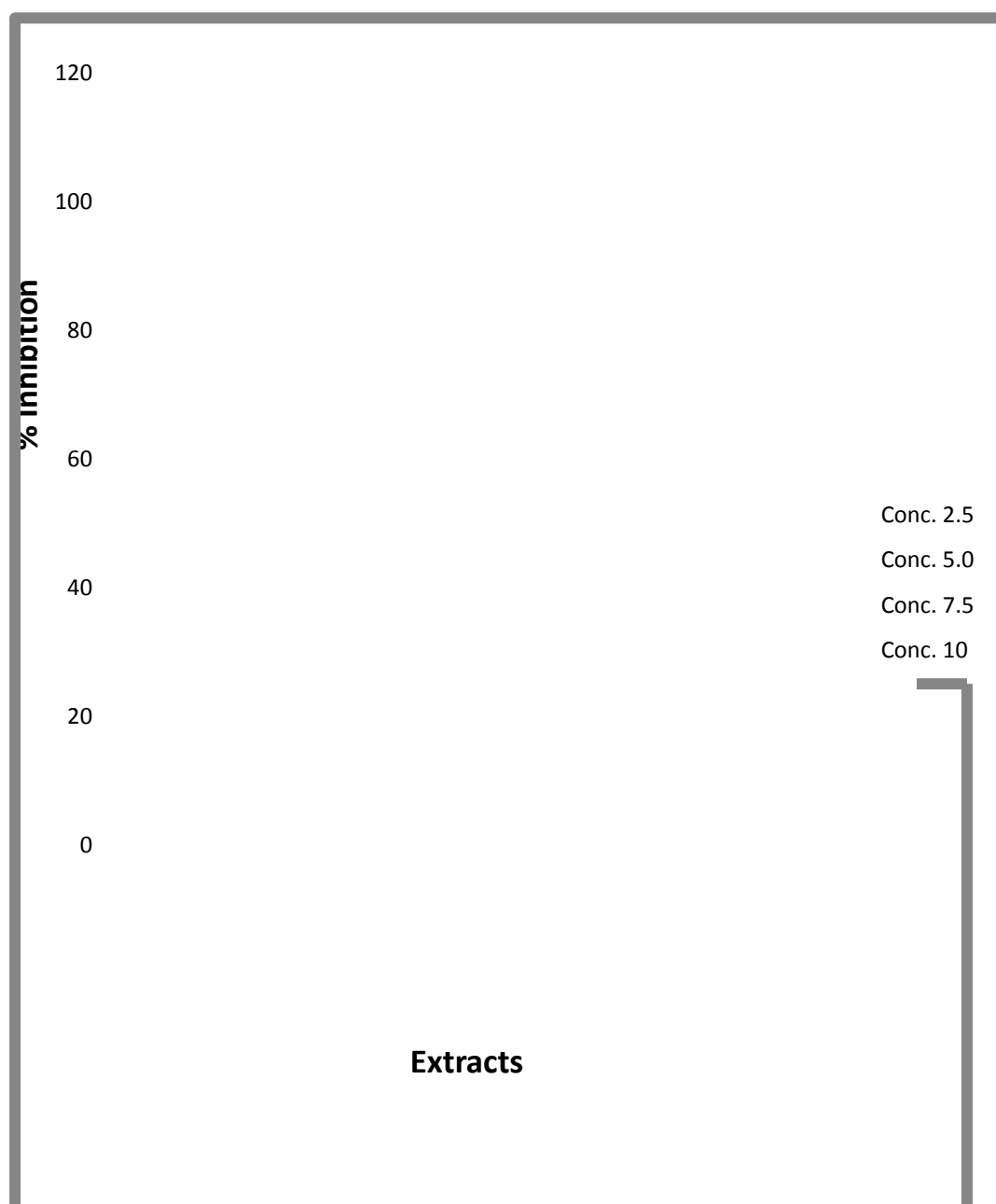
For the comparative effect of *A. indica*, *C. citratus* and their combination on the inhibition of *Aspergillus flavus*, there was a clear synergism between the two plant extract in the control of the mycelia growth of the test fungi. In ethanol, the combined extract gave a very high zone of inhibition with value of 100% at 5% concentration and 92% at other concentrations. These values were significantly ( $P < 0.05$ ) higher than all the values obtained from each of the individual extracts. The highest value from the individual extract was 54% obtained from *A. indica*, hence other values from the individual extracts were less than 54%. In aqueous, synergism also accured between the two extracts in the inhibition of the test organisms. The combined plant extract at 10% concentration nearly gave a complete inhibition of the test fungi with a value of 99%, this is against 18% and 36% inhibition obtained from *C. Citratus* and *A. indica* respectively (Figure 36).



**Figure 36: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Aspergillus flavus***

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Azadi + A-Cymbo** means the mixture of aqueous extract of *Azadirachta indica* and *Cymbopogon citratus*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Cymbo** Means ethanol extract of *Cymbopogon citratus*, **E-Azadi + E-Cymbo** means the mixture of ethanol extract of *Azadirachta indica* and *Cymbopogon citratus*

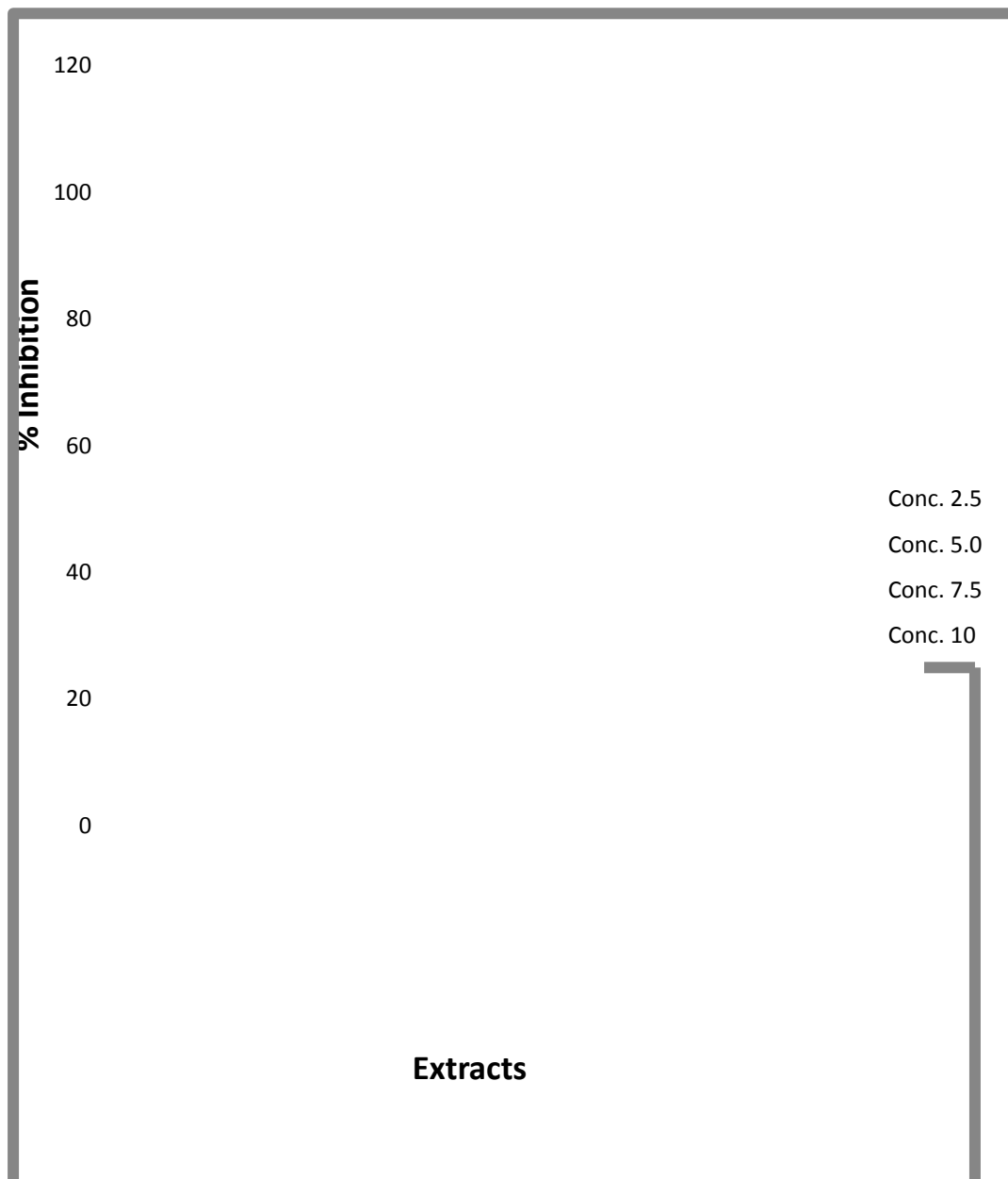
For the effect of *V. amygdalina*, *C. papaya* and their combination on the inhibition of *Aspergillus flavus*, the effect of the combination of the two extracts was more pronounced in ethanol than in aqueous, hence synergism occurred in ethanol extract at all concentrations but only occurred in aqueous at 10% concentration. The combined extracts in ethanol completely inhibited the growth of the test organism at 7.5% and 10% concentrations each while it gave 98.5% inhibition at 5% concentration, these values were significantly ( $P < 0.05$ ) higher than the highest value of 56% inhibition obtained from the individual plant extract. For aqueous extract, the combined plant extract show better inhibitory effect than the individual extract only at 10% concentration, with a value of 99% inhibition, this was significantly ( $P < 0.05$ ) higher than 8% and 37% inhibition obtained from aqueous extract of *C. papaya* and aqueous extract of *V. amygdalina* respectively (Figure 37).



**Figure 37: Comparison between aqueous and ethanol extracts of *Vernonia amygdalina* and *Carica papaya* and their synergistic effects on the inhibition of *Aspergillus flavus***

Key: **A-Vernonia** means aqueous extract of *Vernonia amygdalina*, **A-Carica** Means aqueous extract of *Carica papaya*, **A-Vernonia + A-Carica** means the mixture of aqueous extract of *Vernonia amygdalina* and *Carica papaya*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina*, **E-Carica** Means ethanol extract of *Carica papaya* **E-Vernonia+ E-Carica** means the mixture of ethanol extract of *Vernonia amygdalina* and *Carica papaya*

Synergism occurred between *O. gratissimum* and *V. amygdalina* in the inhibition of the mycelia growth of *A. flavus* although the synergistic effect was higher in ethanol medium than in aqueous medium. The combined extracts in ethanol at all concentrations except at 2.5% gave a high inhibition zone with a value of 92% each and 85% at 2.5% concentration. These were significantly higher than values obtained from the corresponding concentrations of the individual extracts. The highest value recorded from ethanol extract of *V. amygdalian* was 52% while the highest value obtained from ethanol extract of *O. gratissimum* was 37% inhibition. For aqueous, the combined extract gave 62%, 56%, 48% and 31% inhibition at 10%, 7.5%, 5% and 2.5% concentrations respectively, these were significantly ( $P < 0.05$ ) better than all the values obtained from the individual extract at corresponding concentrations (Figure, 38).

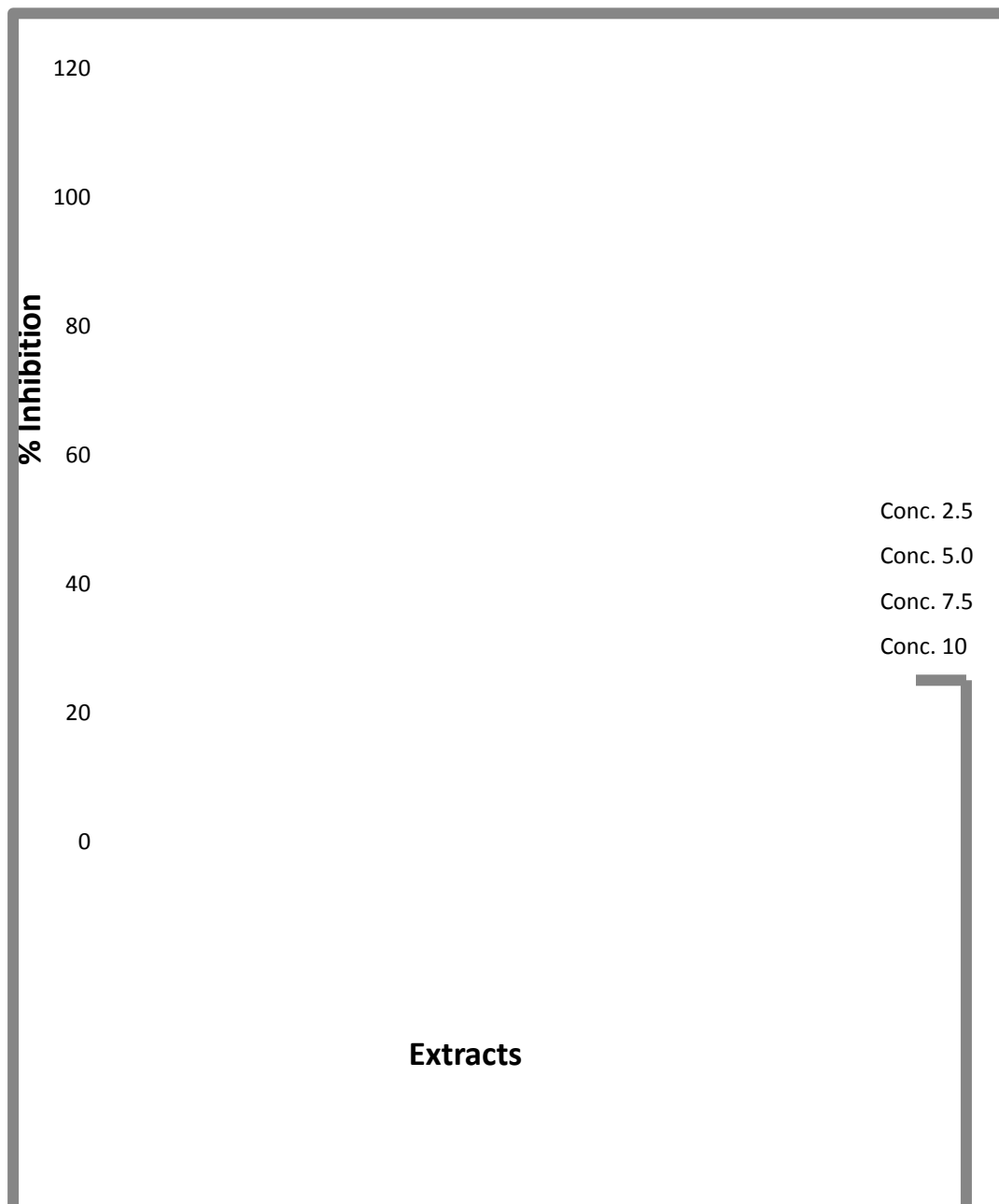


**Figure 38: Comparison between aqueous and ethanol extracts of *Occimum gratissimum* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Aspergillus flavus***

**Key:** **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Occimum + A-Vernonia** means the mixture of aqueous extract of *Occimum grassimum* and *Vernonia amygdalina*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Occimum+ E-Vernonia** means the mixture of ethanol extract of *Occimum gratissimum* and *Vernonia amygdalina*

For the effect of *A. indica*, *V. amygdalina* and their combination on the inhibition of *A. flavus*, the combined extracts showed better inhibition than the individual extract at all concentrations except at 10% concentration in ethanol, hence synergism occurred at all concentrations in both ethanol and aqueous extract except at 10% concentration in ethanol. For ethanol extract, the highest inhibitory effect was recorded from the combined extract at 7.5% and 5% concentrations with 98% and 95% inhibition respectively, these were significantly ( $P < 0.05$ ) better than values obtained from their corresponding concentrations in the individual extract, the value recorded from the combined extract at 10% concentration was 57% this the same with the values obtained from each of the individual extract at 10% concentration, hence there was no synergism at 10% concentration. For aqueous, the combined extract exhibited high synergism at 7.5% and 10% concentration with 58% and 50% inhibition respectively, comparably these values were significantly higher ( $P < 0.05$ ) than the highest value of 38% obtained from the individual extract (Figure 39).

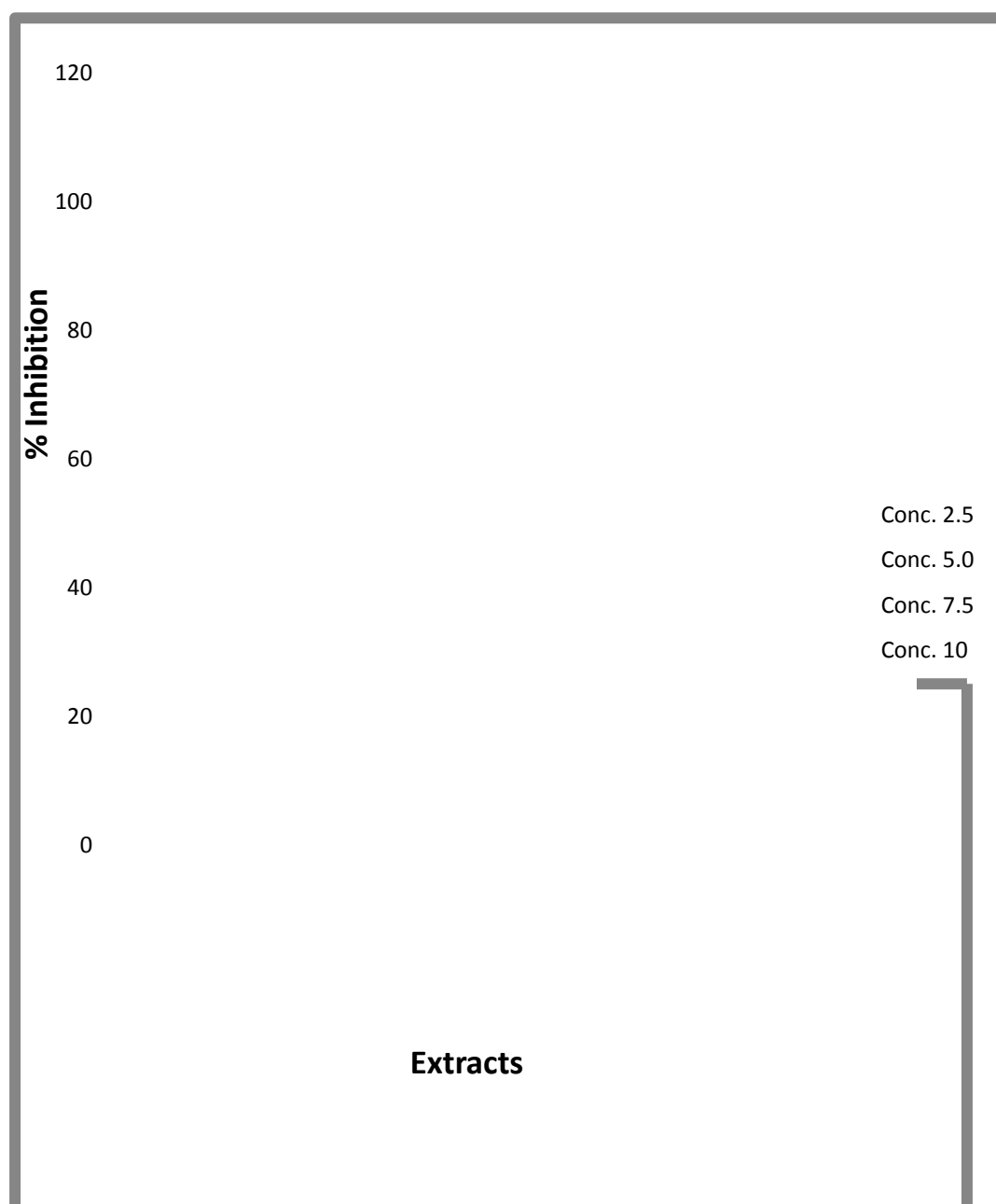




**Figure 39: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Aspergillus flavus***

**Key:** **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Azadi + A-Vernonia** means the mixture of aqueous extract of *Azadirachta indica* and *Vernonia amygdalina*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Azadi+ E-Vernonia** means the mixture of ethanol extract of *Azadirachta indica* and *Vernonia amygdalina*

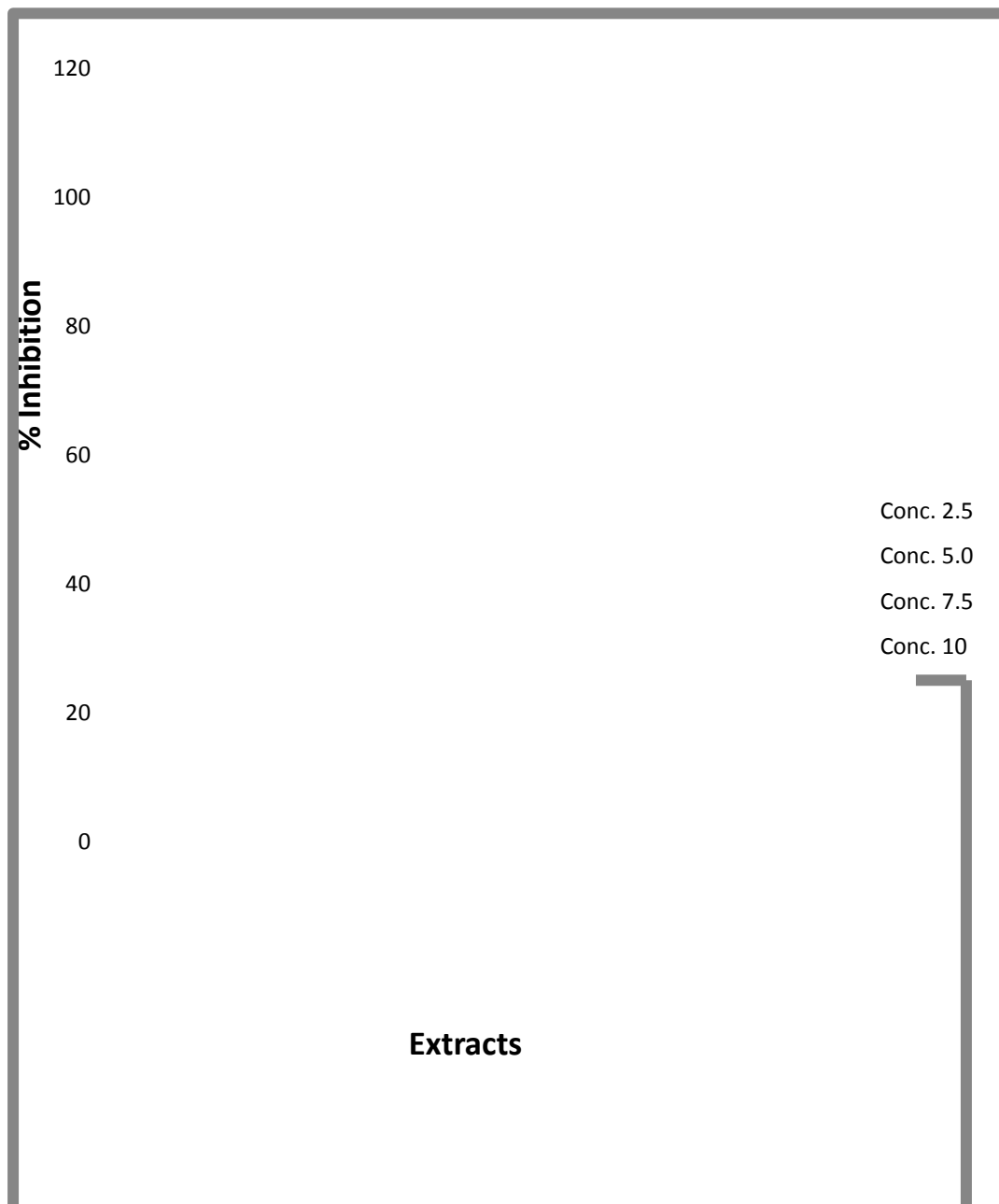
The combination of *C. sinensis* and *O. gratissimum* in the inhibition of *A. flavus* proved to be synergistic in aqueous extract at all concentrations but not synergistic in ethanol extract except at 10% concentration. In ethanol, the combined extract gave a very high inhibition zone at 10% concentration with a value of 92%, this was significantly ( $P < 0.05$ ) higher than values obtained the individual extracts at 10% concentrations each but the values obtained from the individual extract at other concentrations (2.5%, 5.0% and 7.5%) were significantly ( $P < 0.05$ ) higher than values obtained from the combined extract, hence there was no synergism at these concentrations. In aqueous, moderate synergistic effect occurred at all the concentrations, the combined extract yielded values of 47%, 40%, 34% and 32% at 7.5%, 2.5%, 5% and 10% concentrations respectively, these values were significantly ( $P < 0.05$ ) higher than the highest value of 35% obtained from the individual extracts (Figure 40).



**Figure 40: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Occimum gratissimum* and their synergistic effects on the inhibition of *Aspergillus flavus***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-citrus +A-Occimum** means the mixture of aqueous extract of *Citrus sinensis* and *Occimum gratissimum*, **E-Citrus** means ethanol extract of *Citrus sinensis*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Citrus + E-Occimum** means the mixture of ethanol extract of *Citrus sinensis* and *Occimum gratissimum*.

For the effect of *C. papaya*, *C. citratus* and their combination on the inhibition of *A. flavus*, there was prominent synergism between the two extracts in the inhibition of *A. flavus*. Ethanol extract of combined plants gave 99% inhibition at 7.5% and 10% concentrations each and 98% at 2.5% and 5% concentrations each, these were significantly ( $P < 0.05$ ) higher than the highest value of 44% obtained from each of the individual extracts. In aqueous, the combined extract gave 98%, 44%, 32% and 16% at 10%, 7.5%, 5% and 2.5% concentrations respectively, these were significantly ( $P < 0.05$ ) higher than the highest value of 25% obtained from the individual extracts (Figure 41).



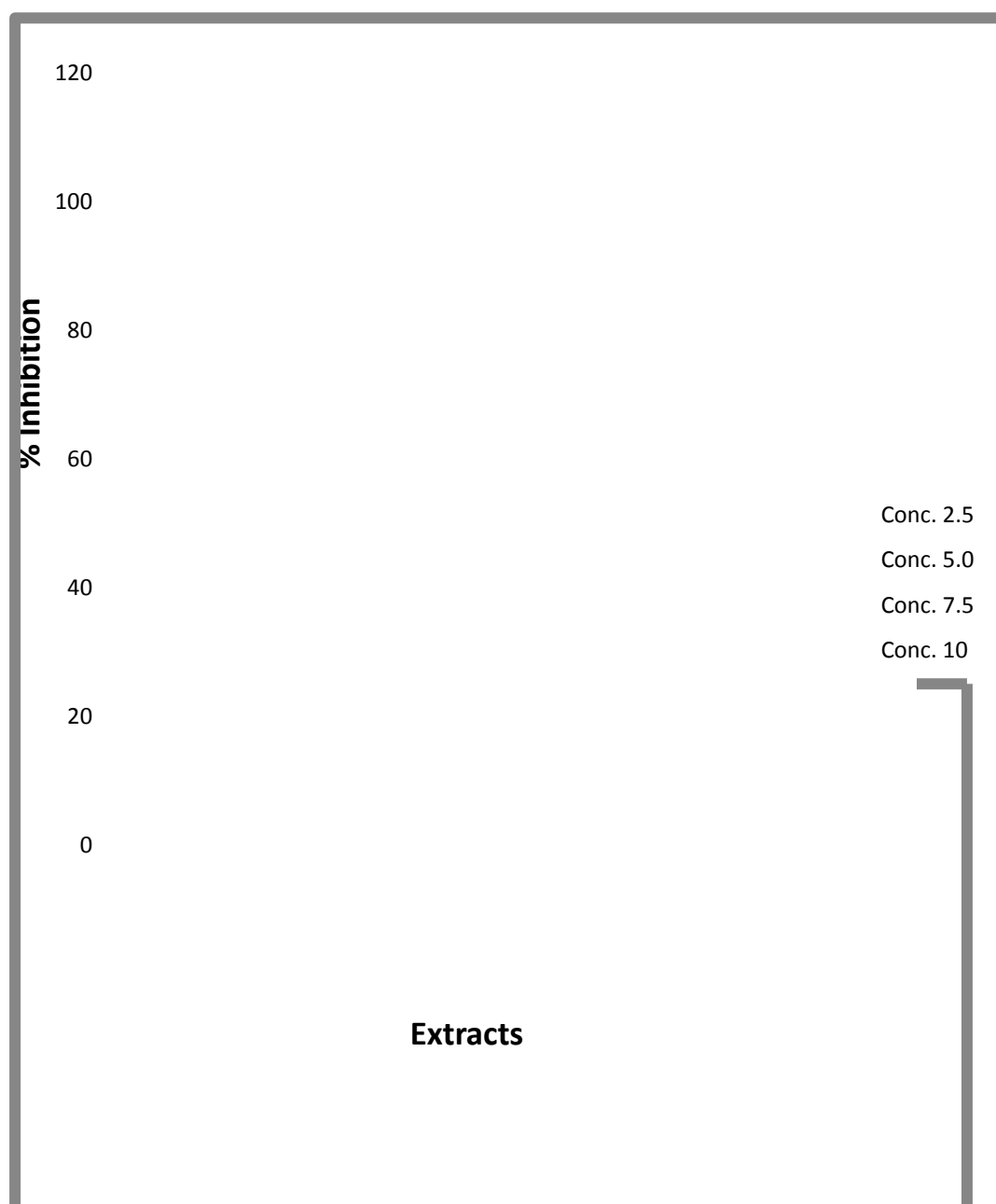
**Figure 41: Comparison between aqueous and ethanol extracts of *Carica papaya* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Aspergillus flavus***

Key: **A-Carica** means aqueous extract of *Carica papaya*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Carica + A-Cymbo** means the mixture of aqueous extract of *Carica papaya* and *Cymbopogon citratus*, **E-Carica** means ethanol extract of *Carica papaya*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus* **E-Carica+ E-Cymbo** means the mixture of ethanol extract of *Carica papaya* and *Cymbopogon citratus*

#### 4.6.6. *Fusarium solani*

The effects of combined extracts on the inhibition of *F. solani* showed to be synergistic both in aqueous and ethanol medium at all concentrations. Although more synergism occurred with increase in concentration.

For the effects of *C. citratus*, *A. indica* and their combination on the inhibition of *F. solani*, synergism occurred at all concentrations and in all extraction media. For ethanol, the combined extract gave its highest inhibition at 7.5% with a value of 98% this was significantly ( $P < 0.05$ ) higher than 46% and 30% inhibition recorded from ethanol extract of *A. indica* and ethanol extract of *C. citratus* respectively at 7.5% concentrations each. In aqueous, the combined extract gave a high inhibition zone with 97%, 96%, 95% and 20% at 10%, 7.5%, 5% and 10% concentrations respectively these were significantly ( $P < 0.05$ ) better than the highest value of 33% obtained from the individual extracts against *F. solani* (Figure 42).

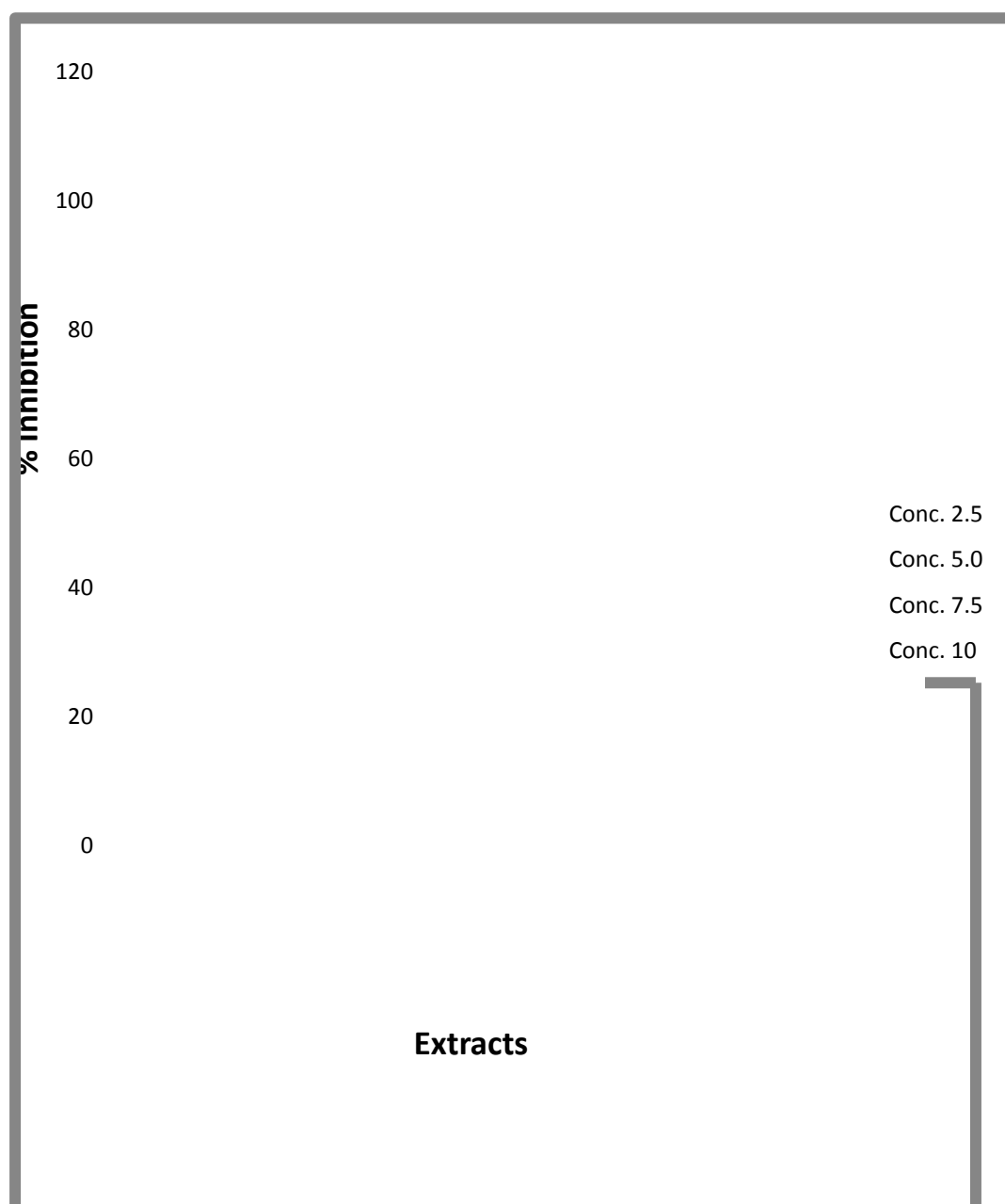


**Figure 42: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Azadirachta indica* and their synergistic effects on the inhibition of *Fusarium solani***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Azadi** Means aqueous extract of *Azadirachta indica*, **A-Citrus + A-Azadi** means the mixture of aqueous extract of *Citrus sinensis* and *Azadirachta indica*, **E-Citrus** means ethanol extract of *Citrus sinensis* **E-Azadi** Means ethanol extract of *Azadirachta indica* **E-Citrus+ E-Azadi** means the mixture of ethanol extract of *Citrus sinensis* and *Azadirachta indica*

The combination of *A. indica* and *C. citratus* against the growth of *F. solani* proved to be synergistic both in aqueous and in ethanol medium. In ethanol, the combined extract gave its highest inhibitory effect at 2.5% and 5% concentrations with 98.5% inhibition each, while at 7.5% and 10% concentration it gave 90% and 94% inhibition respectively. These were significantly ( $P < 0.05$ ) higher than the highest value of 47% obtained from ethanol *A. indica* at 7.5% concentration and 42% inhibition obtained from ethanol extract of *C. citratus*. In aqueous, the synergistic effect was prominent at all concentrations except at 2.5%, the values from the combined extract at 5%, 7.5% and 10% concentrations were 92%, 94% and 94% respectively, these were significantly higher than the highest value of 32% obtained from *A. indica* and the highest value of 21% obtained from *C. citratus* (Figure 43).

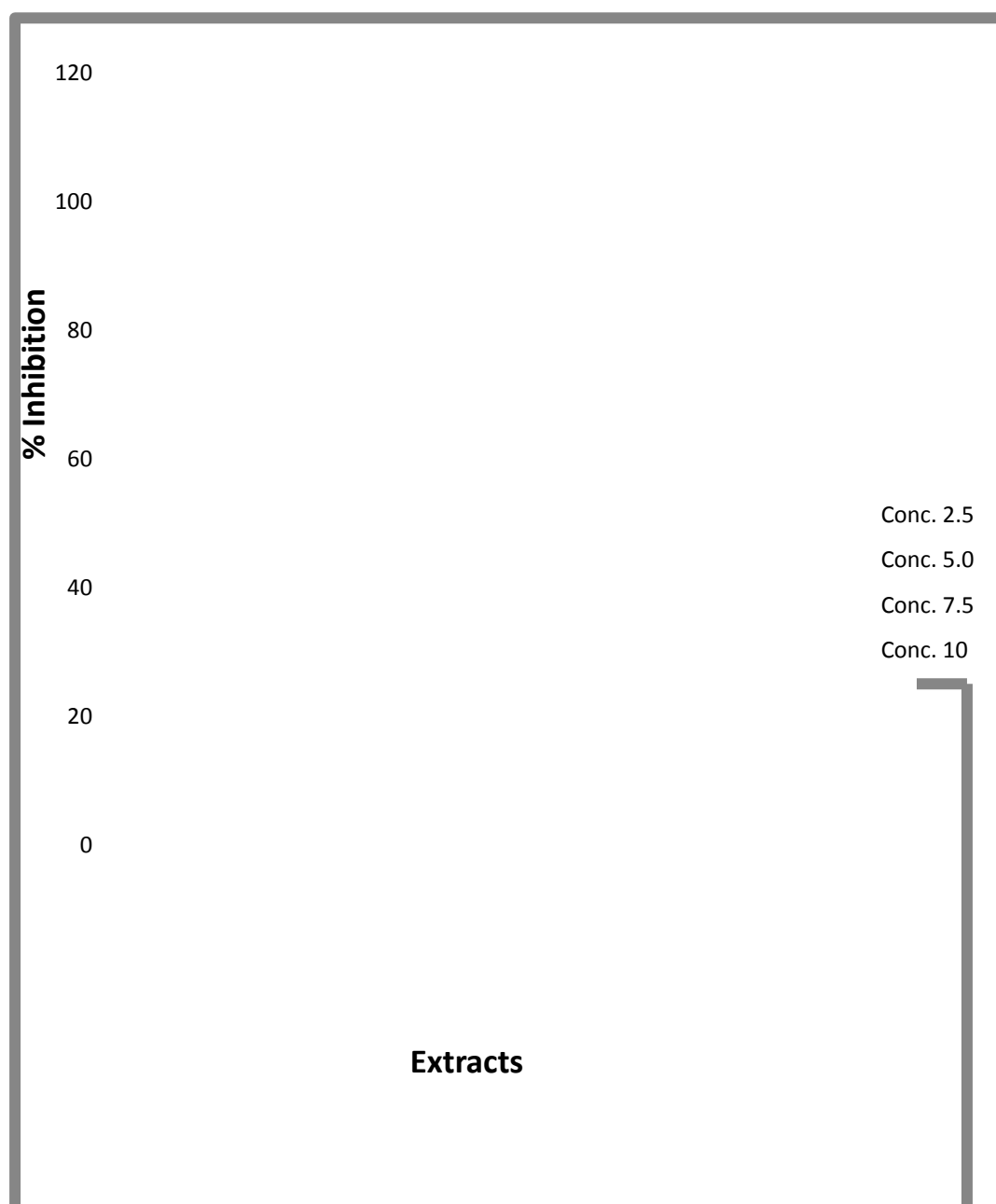




**Figure 43: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Fusarium solani***

Key: **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Cymbo** means aqueous extract of *Cymbopogon citratus*, **A-Azadi + A-Cymbo** means the mixture of aqueous extract of *Azadirachta indica* and *Cymbopogon citratus*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus*, **E-Azadi + E-Cymbo** means the mixture of ethanol extract of *Azadirachta indica* and *Cymbopogon citratus*

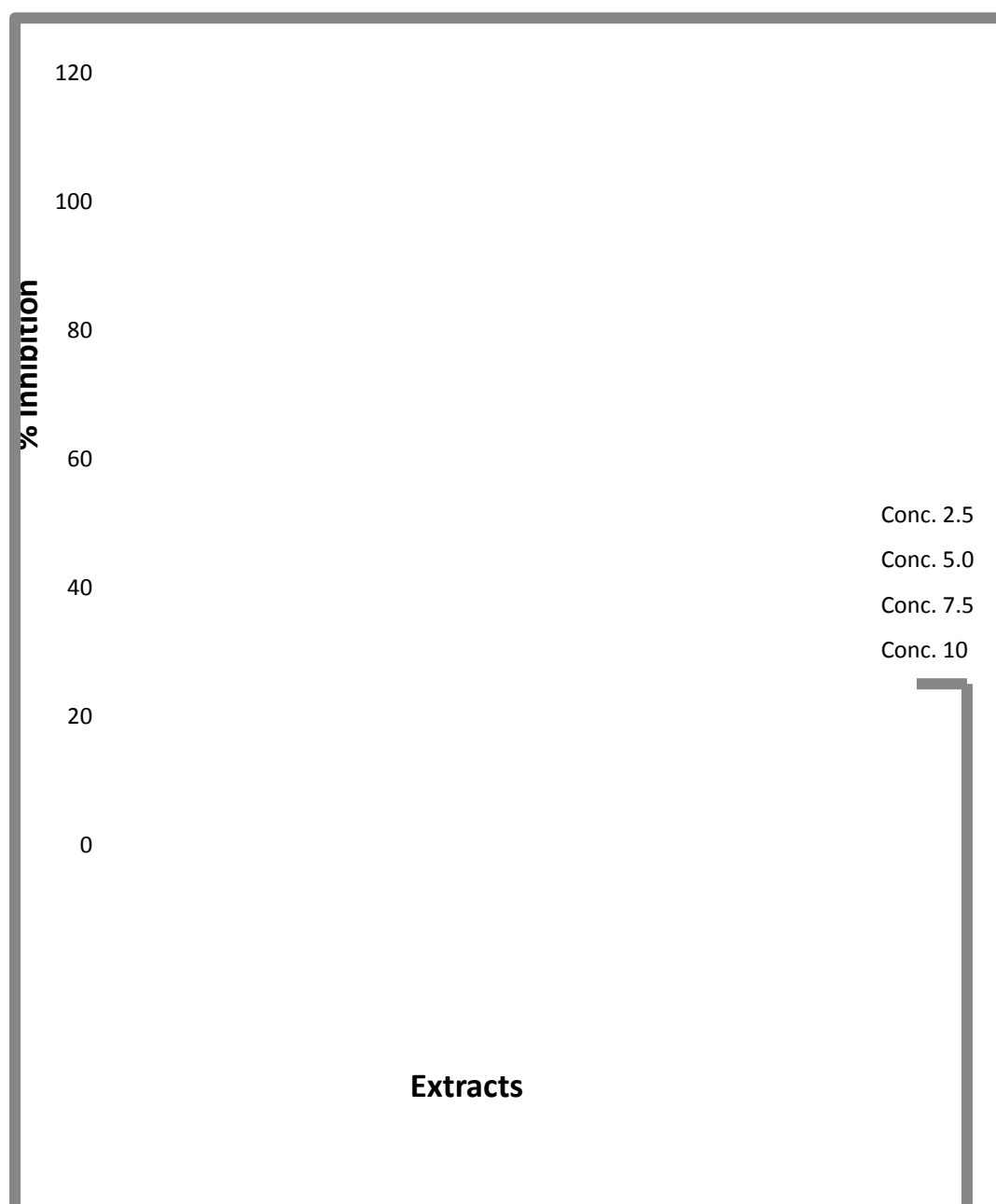
For the effect of *V. amygdalina* and *C. papaya* on the inhibition of the mycelia growth of *F. solani*, the combined extracts in ethanol showed to be more synergistic than the combined extract in ethanol, although synergism occurred in both aqueous and ethanol extracts. With respect to ethanol, the combined extract gave the highest inhibitory effect at 7.5% and 10% concentrations with 98% inhibition and 97% inhibition at 2.5% and 5% concentrations each, these were significantly ( $P < 0.05$ ) higher than the highest value of 39% obtained from ethanol extract of *V. amygdalina* at 10% and 7.5% concentrations each and 22% inhibition obtained from 10% and 7.5% concentration each of ethanol extract of *C. papaya*. For aqueous, the combined extracts proved to be more potent than the individual extract, with the highest value of 97% inhibition obtained at 7.5% concentration this is against the highest value of 29% inhibition obtained from aqueous extract of *V. amygdalina* and 18% inhibition obtained from aqueous extract of *C. papaya* (Figure 44).



**Figure 44: Comparison between aqueous and ethanol extracts of *Vernonia amygdalina* and *Carica papaya* and their synergistic effects on the inhibition of *Fusarium solani***

Key: **A-Vernonia** means aqueous extract of *Vernonia amygdalina*, **A-Carica** Means aqueous extract of *Carica papaya*, **A-Vernonia + A-Carica** means the mixture of aqueous extract of *Vernonia amygdalina* and *Carica papaya*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina*, **E-Carica** Means ethanol extract of *Carica papaya* **E-Vernonia+ E-Carica** means the mixture of ethanol extract of *Vernonia amygdalina* and *Carica papaya*

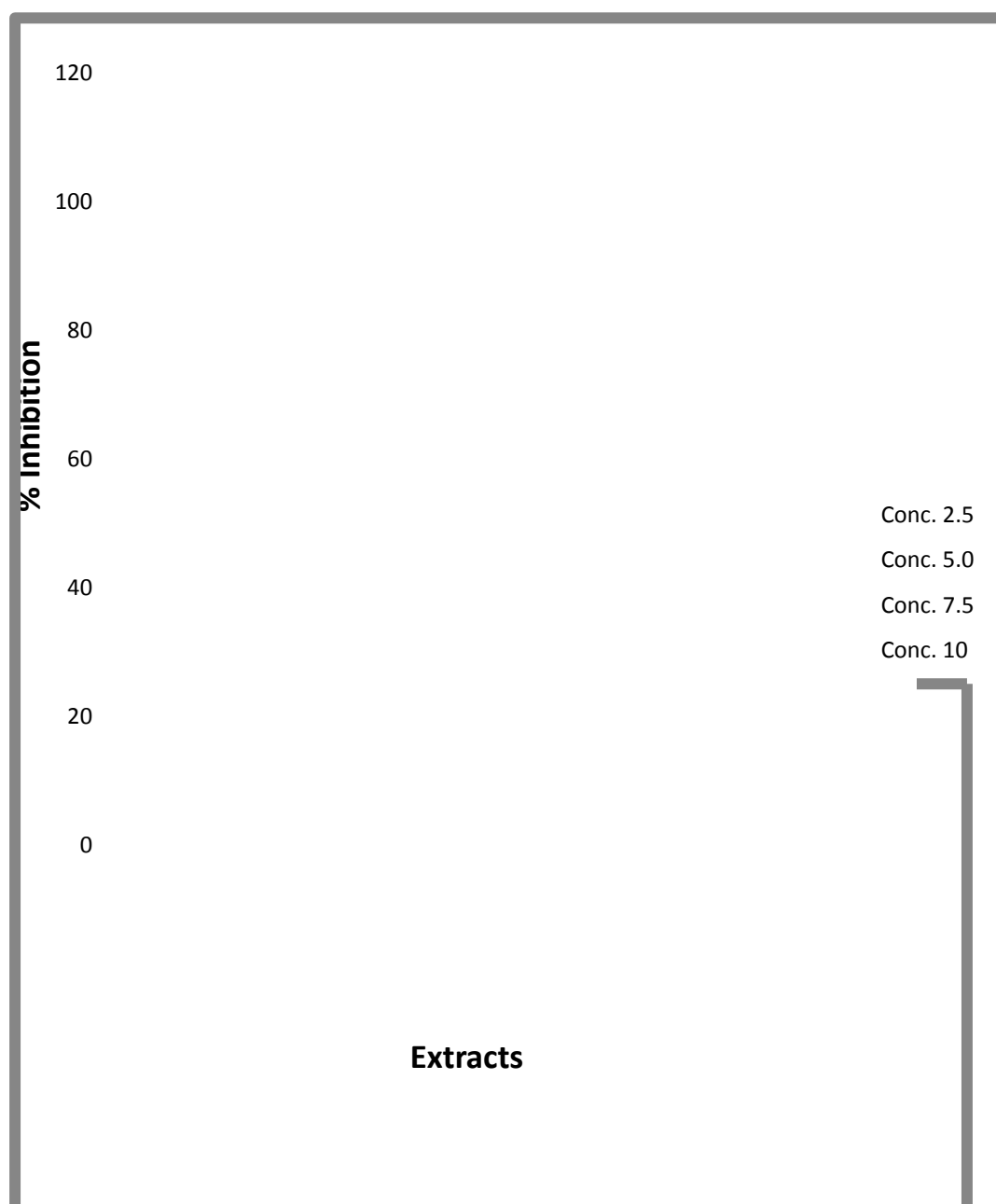
With respect to the effects of *O.gratissimum*, *V. amygdalinai* and their combination on the inhibition of the mycelia growth of *F. solani*, very high synergism occurred in ethanol medium while slight synergism occurred in aqueous extract. In ethanol, the combined extract showed very high degree of inhibition with 94% at all concentration except at 10% concentration where it 96.5%. Each of the individual extracts in ethanol gave values that ranged between 2% to 43%. The highest value of inhibition observed from ethanol extract of *V. amygdalina* was 39% at 7.5% and 10% concentrations each while the highest value obtained from ethanol extract of *O. gratissimum* was 42% at 7.5% and 10% concentrations each. For aqueous, the combined extract did not have synergistic effect against the mycelia growth of *F. solani*. *V. amygdalina* at 7.5% and 10% concentrations gave values that were significantly ( $P<0.05$ ) higher than percentage inhibition recorded from the individual extract, hence there was no synergism with respect to aqueous extract (Figure 45).



**Figure 45: Comparison between aqueous and ethanol extracts of *Occimum gratissimum* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Fusarium solani***

**Key:** **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Occimum + A-Vernonia** means the mixture of aqueous extract of *Occimum gratissimum* and *Vernonia amygdalina*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Occimum+ E-Vernonia** means the mixture of ethanol extract of *Occimum gratissimum* and *Vernonia amygdalina*

For the comparison between the effects of *A. indica*, *V. amygdalina* and their combination on the inhibition of the mycelia growth of *F. solani*, synergism occurred between the extract both in aqueous and in ethanol. In ethanol, the combined extract proved to be more potent than the individual extract at all concentrations except at 2.5%. The combination of the two extract gave a very high inhibition zone with values of 93%, 94% and 95% at 10%, 7.5% and 5% concentrations respectively. These were significantly ( $P < 0.05$ ) higher than the highest values of 39% and 46% obtained from *V. amygdalina* and *A. indica* respectively all at 7.5% concentration. For aqueous, the combined extract proved to be better than the individual extracts at all concentrations, the inhibition percentages obtained from the combined extracts was 96%, 95%, 94% and 93% at 10%, 7.5%, 5% and 2.5% concentrations respectively these were significantly higher than values obtained from each of the individual extract at corresponding concentrations, hence synergism occurred between *A. indica* and *V. amygdalina* in the control of *F. solani* (Figure 46).

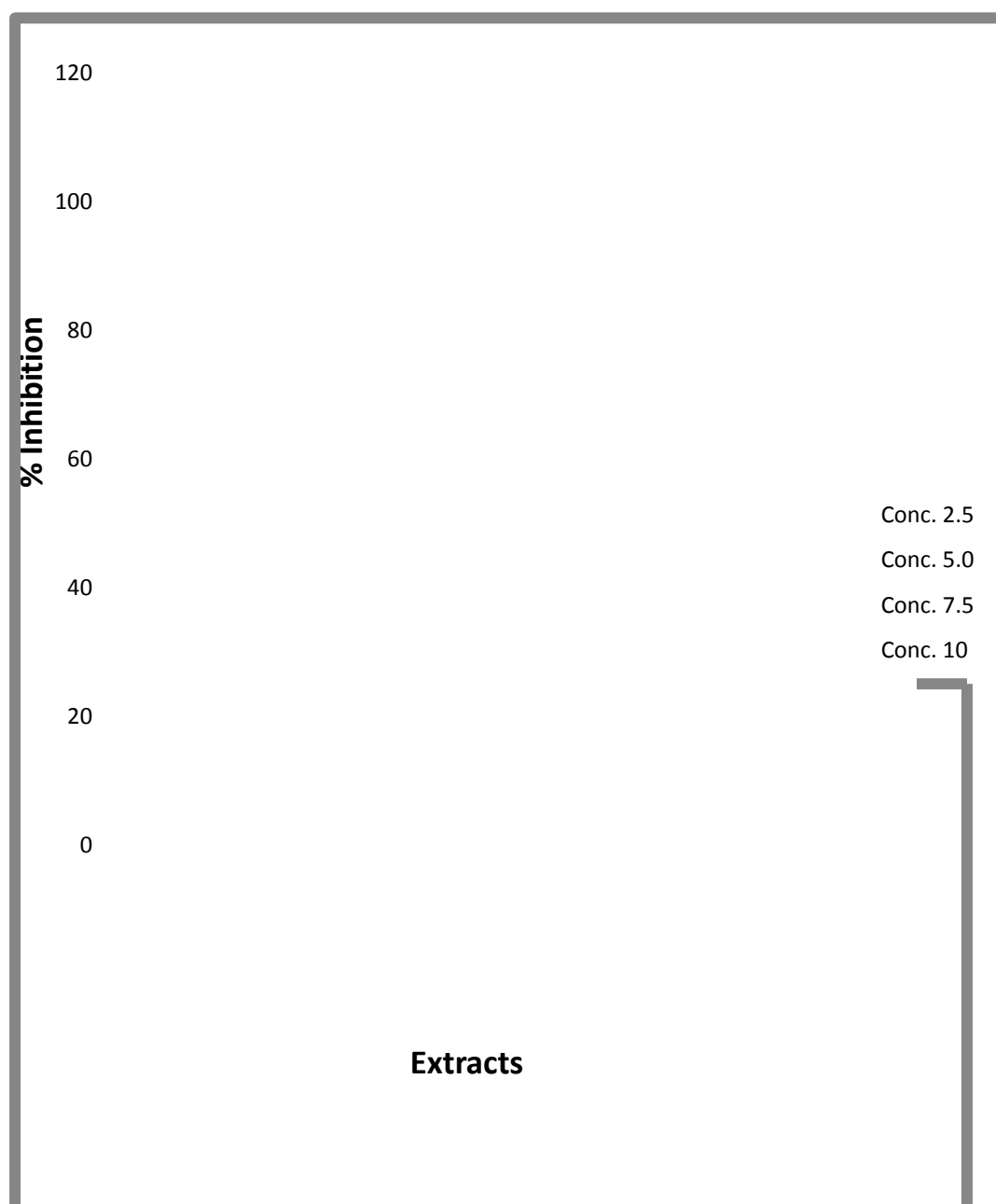


**Figure 46: Comparison between aqueous and ethanol extracts of *Azadirachta indica* and *Vernonia amygdalina* and their synergistic effects on the inhibition of *Fusarium solani***

**Key:** **A-Azadi** means aqueous extract of *Azadirachta indica*, **A-Vernonia** Means aqueous extract of *Vernonia amygdalina*, **A-Azadi + A-Vernonia** means the mixture of aqueous extract of *Azadirachta indica* and *Vernonia amygdalina*, **E-Azadi** means ethanol extract of *Azadirachta indica*, **E-Vernonia** means ethanol extract of *Vernonia amygdalina* **E-Azadi+ E-Vernonia** means the mixture of ethanol extract of *Azadirachta indica* and *Vernonia amygdalina*

Synergism occurred between *C. sinensis* and *O. gratissimum* in both ethanol and aqueous in the control of *F. solani*, the degree of the synergism varies with concentration of the extract. For ethanol, the combined extract gave a very high rate of inhibition at 2.5% and 7.5% concentrations with values of 90% and 70% respectively. These were significantly ( $P < 0.05$ ) higher than the highest value of 41% obtained from *O. gratissimum* at 7.5% and 10% concentrations each; they were also significantly ( $P < 0.05$ ) higher than the highest value of 30% inhibition obtained from *C. citratus* at 7.5% concentration. For aqueous, there was prominent degree of synergism between the two plant extract, hence the values obtained from the combined extracts at all concentrations were significantly ( $P < 0.05$ ) higher than the values obtained from the individual extracts at corresponding concentrations. The values obtained from the combined extracts was 91%, 72%, 18% and 40% at 10%, 7.5%, 5% and 2.5% concentrations respectively, these were significantly higher than the highest value of 8.5% obtained from the individual extracts (Figure 47).

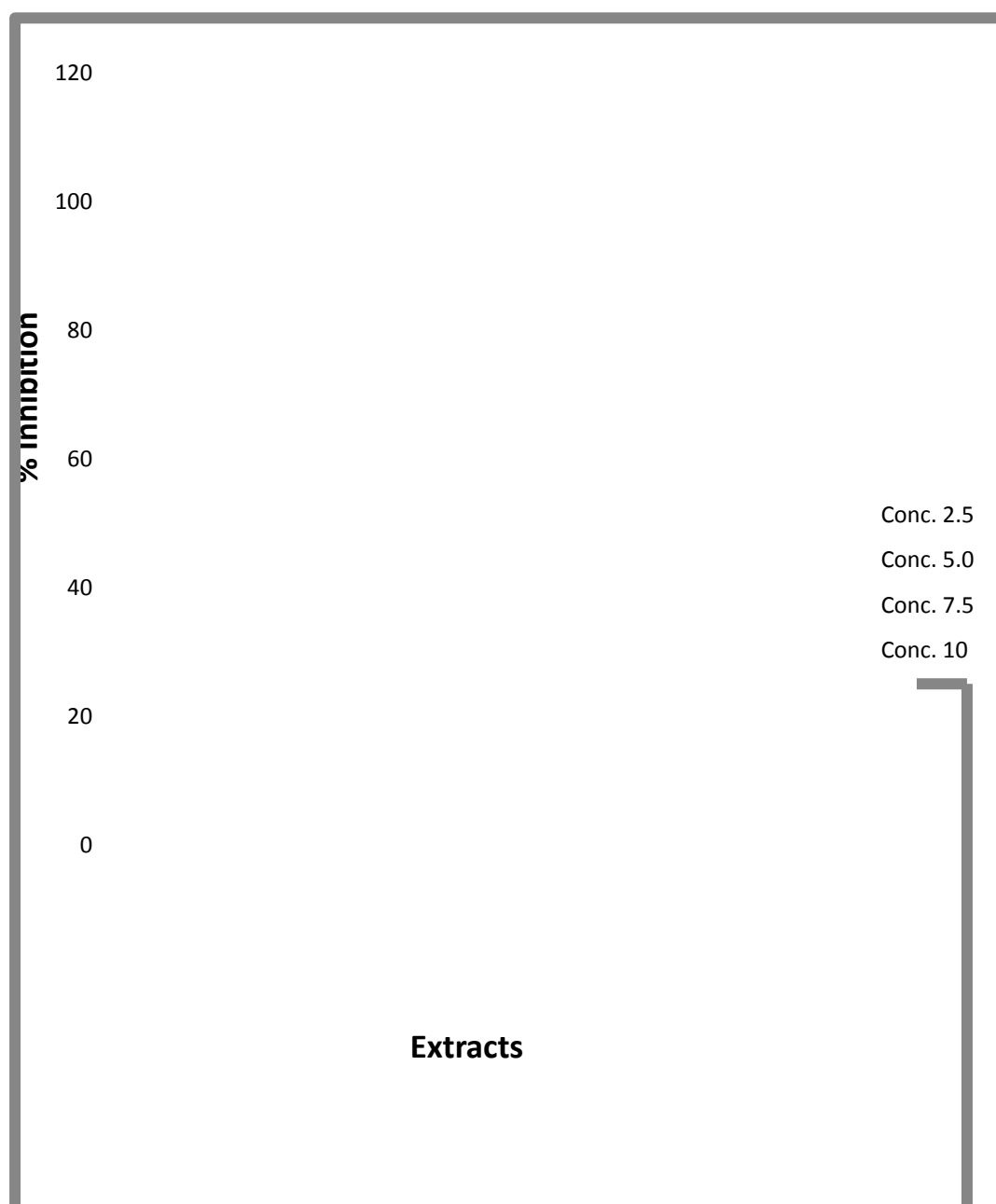




**Figure 47: Comparison between aqueous and ethanol extracts of *Citrus sinensis* and *Occimum gratissimum* and their synergistic effects on the inhibition of *Fusarium solani***

Key: **A-Citrus** means aqueous extract of *Citrus sinensis*, **A-Occimum** means aqueous extract of *Occimum gratissimum*, **A-citrus +A-Occimum** means the mixture of aqueous extract of *Citrus sinensis* and *Occimum gratissimum*, **E-Citrus** means ethanol extract of *Citrus sinensis*, **E-Occimum** means ethanol extract of *Occimum gratissimum*, **E-Citrus + E-Occimum** means the mixture of ethanol extract of *Citrus sinensis* and *Occimum gratissimum*.

With respect to the effect of *C. papaya*, *C. citratus* and their combination on the inhibition of *F. solani*, the extracts showed synergism especially at 7.5% and 10% concentration. In ethanol, the combine extract gave a very high inhibition across all the concentrations with values of 76%, 82%, 74% and 50% at 10%, 7.5%, 5% and 2.5% concentrations respectively. These values were significantly ( $P < 0.05$ ) higher than the highest value of 42% obtained from ethanol extract of *C. citratus* at 10% concentration. In aqueous, synergism only occurred at 7.5% and 10% concentrations with inhibition values of 94% and 93% respectively this is against the highest value of 22% obtained from the individual extracts, hence synergistic effect occurred at 7.5% and 10% concentrations because at other concentrations (2.5% and 5%) the values obtained from the individual extract was significantly higher than the values obtained from the combined extracts (Figure 48).



**Figure 48: Comparison between aqueous and ethanol extracts of *Carica papaya* and *Cymbopogon citratus* and their synergistic effects on the inhibition of *Fusarium solani***

Key: **A-Carica** means aqueous extract of *Carica papaya*, **A-Cymbo** Means aqueous extract of *Cymbopogon citratus*, **A-Carica + A-Cymbo** means the mixture of aqueous extract of *Carica papaya* and *Cymbopogon citratus*, **E-Carica** means ethanol extract of *Carica papaya*, **E-Cymbo** means ethanol extract of *Cymbopogon citratus* **E-Carica+ E-Cymbo** means the mixture of ethanol extract of *Carica papaya* and *Cymbopogon citratus*

#### **4.7. Phytochemical Analysis of Plant Materials**

Qualitative and quantitative analysis were carried out to determine the presence and quantity of phytochemicals in the mixed samples of plant materials.

##### **4.7.1. Qualitative phytochemical screening**

Phytochemicals of interest (phytate, flavonoid, alkaloid, saponin, tannins, oxalate and phenol) were tested. The result of the qualitative screening showed the presence of all the phytochemicals in all the plant extracts mixture (Table 10).

**Table 10: Qualitative phytochemical analysis of the combined plant extracts**

Combined plant extracts							
Phytochemicals	N + B	O + S	P + L	N + L	B + P	O + N	S + B
Phytate	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Oxalate	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+

**Key:** N + B= Neem and Bitter leaves, O + S= Orange bark and Scent leaves,  
P + L= Paw paw and Lemon, N + L= Neem and Lemon, B+P= Bitter leaves and Paw paw, O  
+N= Orange bark and Neem, S +B= Scent leaves and Bitter leaves

#### 4.7.2. Quantitative phytochemical screening

The quantitative yield showed that the quantities of phytochemicals in these plant materials ranged from 0.08% and 14.9%. The combination of Pawpaw and Lemon grass (P+L) gave the highest quantity of phytate with a values of 6.32%, next to this was 4.22% recorded from the mixture of Orange and Scent leave (O+S), while the least in phytate was 0.66% observed from the mixture of Neem and Bitter leaf (N+B). For flavonoid the highest value of 10.01% was observed from the mixture of Orange and Scent leaves (O+S) next were 7.84% and 7.91% from Neem/Bitter leaves and Orange/Neem respectively, least in flavonoid was observed from the mixture of Scent leave and Bitter leave. For alkaloid, the highest yield was observed from the mixture of Orange/Neem (6.19%) while the least was Pawpaw/Bitter leaves (2.19%). For saponin the highest values of 7.34% was obtained from the mixture of Orange/Scent leaves followed by 4.90% observed from Neem/Lemon and the least in saponin yield was Scent leave/Bitter leave (2.00%). The quantity of Tannins in Neem/Orange is 14.9% this was the highest followed by Neem/Lemon (10.12%) while the least quantity of Tannins was observed in Scent leave/Bitter leave (0.08%). More oxalate was detected in Orange/Neem (8.67%), the least in oxalate was Bitter leaves/Pawpaw (0.60%). Test for phenol depicted that Orange/Scent leaves with a yield of 5.92% was highest, next to it being Scent leaves/Bitter leaves (2.21%) while the least was Orange/Scent leaves (5.92%) (Table 11).

Table 11: Phytochemical compositions of combined plant extracts (%)

Plant extracts	Phytochemicals (%)						
	Phytate	Favonoid	Alkaloid	Saponin	Tannins	Oxalate	Phenol
N + B	0.66	7.84	4.60	2.04	1.42	0.74	1.75
O + S	4.22	10.01	5.12	7.34	9.97	4.06	5.92
P + L	6.32	2.86	2.52	4.28	3.41	1.54	0.98
N + L	1.09	6.34	3.76	4.90	10.12	4.60	0.08
B + P	0.44	1.34	2.19	4.34	0.76	0.60	0.45
O + N	5.58	7.91	6.19	3.11	14.9	8.67	1.09
S + B	1.32	0.44	2.22	2.00	0.08	1.43	2.21

**Key:** N + B= Neem and Bitter leaves, O + S= Orange bark and Scent leaves,  
P + L= Paw paw and Lemon, N + L= Neem and Lemon, B+P= Bitter leaves and Paw paw, O  
+N= Orange bark and Neem, S +B= Scent leaves and Bitter leaves

## CHAPTER FIVE

### DISCUSSION

Six species of fungi (*Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Fusarium solani*, *Aspergillus niger*, *Penicillium* spp and *Aspergillus flavus*) were isolated from the rotten yam tubers. This agrees with the report of Dooshima *et al.* (2015) who reported the presence of all the organisms listed above except *A. flavus*. There is disagreement in the findings of this research with those of some. Ogaraku and Usman (2008) who in addition to *A. flavus*, *A. niger*, *Rhizopus* spp and *Fusarium oxysporium* reported the presence of *Sclerotia rolfsi* and *Rhizoctonia* spp but did not report *Botryodiplodia theobromae*. The most common fungal isolates were *Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Fusarium solani*, which does not agree with the findings of Dooshima *et al.* (2015) reported highest incidence of *Aspergillus niger*. The most pathogenic organism in the present study was *Botryodiplodia theobroma* while the least was *Penicillium* spp, in contrast with the findings of Okigbo *et al.* (2015) on *Dioscorea rotundata* from Awka, Anambra Stated that the most pathogenic was *Aspergillus niger* while the least virulent was *Fusarium solani*.

The result of this research showed that *A. indica* and *V. amygdalina* inhibited the growth of the test organisms more than other extracts screened for their inhibition against the various fungi responsible for post harvest spoilage of yam. This is similar with the findings of Owolabi *et al.* (2017) who reported that *Azadirachta indica* stem bark ethanolic extract and *Azadirachta indica* leaf water extract were effective against all four test organisms. The findings of this work slightly differ with the result of Okpara *et al.* (2014) who reported that *O. gratissimum* is more active than *V. amygdalina* in the control of pathogenic microorganisms of yam rot.

It was noted from this research that ethanolic extract showed greater effect in the inhibition than aqueous extract, this suggests that water used in the extraction process was



probably not able to dissolve all the principle compounds present in the plants which are contained in the ethanol extracts. In other words, it can be said that alcohol is the better solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts. The higher inhibition of ethanol extracts observed in this research agrees with the result of Ekwenye and Elegalam (2005) who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence it liberates the active principles required for fungal inhibition. This is also in tandem with the result of Okigbo *et al.* (2009) who reported that ethanol extracts of *Allium sativum*, *Garcinia kola* and *Azadirachta indica* were more effective than aqueous extracts. The differences in the efficacy of the different extraction medium can also be linked to the susceptibility of each of the test fungi to different concentrations of the extracts, this also agrees with the findings of some workers using some plant materials on some microorganisms associated with the rot of cocoyam, potatoes and other root and tuber crops apart from yam (Onifade, 2002; Okigbo and Nmeko, 2005; Okigbo and Odurukwe, 2009) although the reports of several other researchers do not agree with the above axiom. For instance Tafesse *et al.* (2006) reported that there was no significant difference in the effects of aqueous extracts and their ethanol counterparts.

Most tested mixtures of plant extracts showed varying degrees of antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Penicillium* Spp, *Botryodiplodia theobromae* and *Rhizopus stolonifer* which may reflect the presence of biological active ingredients that inhibit fungal growth and proliferation.

The present observations showed that the aqueous extracts of the following mixtures of plant materials: *A.indica* (Neem)/*C.citratus* (Lemon grass), *C.sinensis* (Orange)/*A.indica* (Neem) and *A.indica* (Neem)/*V.amygdalina* (Bitter leaf) were highly effective against the mycelia growth of *F.solani*. This indicates that all the aqueous extracts in combination of Neem were more

potent than those that do not have Neem as a component, this agrees with the results of Okigbo *et al.* (2015) who reported that Neem extract was highly effective against the mycelia growth of *F.solani* but differs slightly with the result of Okigbo *et al.*, (2009b) who reported a moderately effective inhibition on the mycelia growth of *F.solani* with Neem. The effectiveness of the different plant mixtures against *F.solani* differed with extraction medium. The most effective combination of ethanol extracts of plant materials were Neem/Lemon grass, Bitter leaf/Pawpaw, Scent leaf/Bitter leaf and Neem/Bitter leaf. This suggests that the biological active ingredients in Bitter leaf dissolves better in ethanol than in aqueous while that of Neem dissolves better in aqueous. All the extract combinations both in aqueous and in ethanol exhibited the best inhibitory effect against *F.solani* at 7.5% extract concentration this does not agree with the reports of several workers who reported the best inhibitory effects at 10 extract concentrations. For instance Okigbo *et al.* (2013, 2014).

Effects of aqueous mixture of plant materials on the inhibition of *Rhizopus stolonifer* varied with the plant materials used and the concentrations of the mixtures. Pawpaw/Lemon grass and Neem/Bitter leaf showed a very high synergistic effect, this is in sharp contrast with the result of a study conducted by Okigbo *et al.*(2015) on post- harvest rot of cocoyam who reported that Pawpaw alone or its mixture with other plant materials had a very limited effect on the growth of *R.stolonifer*. For ethanol extracts, Bitter leaf/Pawpaw, Scent leaf/Bitter leaf and Pawpaw/Lemon grass gave very high inhibitory effect. The difference in the efficacy of the plant materials in different solvent can be linked to the solubility of the biological active ingredient contained in these plants to different solvents. Water and ethanol have molecular weights that are far apart; this no doubt affects the solubility of materials dissolved in them. Furthermore, the result of this study revealed that the higher the concentrations of ethanol and aqueous extracts, the more the inhibition, hence the inhibitory effects increase with increase in concentration

(10% > 7.5% > 5.0% > 2.5%). This is in tandem with the results of several researchers, for instance the report of Okigbo *et al.* (2017) on *Rhizopus stolonifer* as one of the pathogens associated with cocoyam rot, confirmed that the higher the concentrations of extracts, the more their inhibition on *Rhizopus stolonifer*. Commercial fungicide used in this research as the positive control was not as effective as the mixture of plant extracts at 7.5% and 10% concentrations, this disagrees with the results of many researchers who reported that fungicide was better as compared to the effects of one plant materials (not mixture) on *Rhizopus stolonifer* (Okigbo *et al.*, 2009a; Okigbo, *et al.*, 2009b)

*Botryodiplodia theobromae* was sensitive to the type of solvent, concentration and mixture of plant materials. The aqueous mixture of Neem/Bitter leaf and Orange/Scent leaf inhibited the growth of *B.theobromae* more than the mixture of other plant materials. Generally, 7.5% extract concentration was more effective than other concentrations; this suggests that concentration more or below 7.5% will not produce the highest inhibitory effect. All or most ethanolic extract mixtures proved to be better than aqueous on *Botryodiplodia theobromae* except Neem/Bitter leaf and Pawpaw/Lemon grass, this does not completely agree with the results of Amienyo and Ataga (2007) on the occurrence and control of fungal pathogens of potato with plant extracts. They stated that Pawpaw and lemon even at a very high concentration do not show any significant inhibition on the growth of *Botryodiplodia theobromae*. Synthetic fungicide used gave moderate inhibitory effect on *Botryodiplodia theobromae*, the efficacy was low than the effects of combined plant materials at 7.5% and 10% concentrations, this does not support the findings of so many researchers who reported that synthetic fungicides such as mancozed and grisovid were more active in the inhibition of the mycelia growth of *Botryodiplodia theobromae* (Okigbo *et al.*, 2009a; 2009b) than plant materials/botanicals.

Results obtained from the effects of various of plant materials at different concentrations on *Penicillium* spp showed that the mixture of Neem/Lemon grass, Bitter leaf/Pawpaw and Orange/Neem were better inhibitors than other combinations of plant materials. It was also observed that ethanol extract of Bitter leaf/Pawpaw and Scent leaf/Bitter leaf were also very good inhibitors of *Penicillium* spp. This agrees with the recent findings of Arikpo *et al.* (2013) who reported that Neem and bitter leaf has high antifungi activity. Both aqueous and ethanol extracts best inhibited the growth of these organisms at 10% extract concentration, this is in line with the findings of Al-Manhel and Niamah (2015) but in sharp contrast with the reports of Mada *et al.* (2013). Synthetic fungicides used for the microbial inhibition of *Penicillium* spp proved to be very effective.

The result of this study revealed that the combination of all the test plants in both solvents (water and ethanol) had reasonable effect on the inhibition of *A.flavus* at 7 days after inoculation. Although the ethanol extract was more potent than aqueous with the mixture of Orange/Neem exhibiting the best inhibition for aqueous, this reinforces the points made by several workers on the efficacy of Neem extract alone or in combination with other materials in the inhibition of *A. flavus* (Ayly *et al.*,2013). In ethanol all the plant extract combination except the mixture of Orange/Scent leaf significantly inhibited the growth of *A.flavus* but relatively higher degrees of inhibition was observed at 7.5 and 10% extract concentrations. Nevertheless, the inhibition observed in this research could be linked to the synergistic effects of the different plant materials mixed. This is in line with the report of Al-Manhel and Niamah (2015) who stated that the potency of plant on the inhibition of microbial growth is enhanced when the different plant materials are mixed together.

It was observed from the findings of this study that ethanol extracts of all the plant extract mixtures did relatively better in inhibiting the growth of *A. niger in vitro* than aqueous extracts.

The mixture of Scent leaf/Bitter leaf proved to be more effective than other extract mixtures in both aqueous and ethanol. The result of this study also revealed that the more the concentrations of the various plant mixtures, the more the inhibitory effect on *A. niger*. In other words, concentration of the extract is a major factor that determines their rate of inhibition on *A.niger*. This completely agrees with the documentations of so many researchers (Mahmoud *et al.*, 2011; Shubhi *et al.*, 2010; Okigbo *et al.*,2009a;) who variously stated that the effects of extracts on *A. niger* gradually increased with concentration, with the highest concentration given the strongest inhibition, but disagrees with the reports of Bassey *et al.* (2012) who stated that concentration of extract has little or no effect on the inhibition of *A. niger*. The synthetic fungicide used also proved very effective against *A.niger*. It had more effect on the inhibition of this organism than all the plant extract mixtures in both aqueous and ethanol except in ethanol extract of Scent leaf/Bitter leaf.

The individual extract and their combinations demonstrated either synergistic, additive or indifferent interaction effect against the test. The combined extracts were better inhibitor than the individual extracts at corresponding concentrations.

In this research, the plant extracts had different synergistic ability in the inhibition of the growth of fungal organisms isolated from yams with symptoms of post-harvest rot depending on the extraction medium, concentrations of the extracts and the test organisms. Most of the extracts combined together showed better inhibition against the test organisms than when used singly. This result is in consonance with the earlier reports of several researchers but on different fungal organisms (Okigbo *et al.* 2009), hence the combination of these plant parts have the potential of protecting mechanically injured yam tubers against pathogenic fungal organisms. Biological active principles in plants have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are applied concurrently they give

better result (Reddy *et al.*, 2004). It has been documented that one of the effective means of overcoming microbial resistance is restoration of antimicrobial activity through the synergistic action of antimicrobial materials from natural and botanical agents (Saravanan *et al.*, 2010). Synergism between bioactive plant extracts is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) (Adwan and Mhanna, 2008). The differences in inhibition observed across the mixture of plant extracts can also be attributed to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process. The synergistic effect may be due to formation of certain complexes which become more effective in the inhibition of particular microorganisms when mixed together. Similar conclusions were drawn by Zafar *et al.* (2010) who studies the synergistic effect of extracts on pathogenic organisms.

## CONCLUSION

The inference of this research work depicted that post-harvest rot of yam is chiefly caused by pathogenic fungal organisms. It was also observed that the radial growth (*in vitro*) of the entire test organisms were greatly inhibited by all the plant extracts mixtures tested at varying degrees. This is an indication that fungitoxic compounds abound in the plant materials. The sensitivity of the six test fungi differs with the mixture of the plant materials. In other words, some fungi that were inhibited by a particular mixture of plant materials were not affected by other mixtures.

All the combination of extracts that had *A. indica* and *V. amygdalina* as a component did better than others in aqueous. While relatively higher susceptibility of all the organisms was observed at 7.5% and 10% extract concentration. Comparably, ethanol extract was more

efficacious with regards to the matrices of parameter studied. All the plant extracts depicted high degree of synergism against all the test organisms at varying degrees depending on extraction medium and concentration of the extract. The results obtained from phytochemical screening of the different plant extracts confirm that these plant materials are rich in biological substances that have the great potential of controlling pathogenic organisms responsible for the rot of root and tuber crops.

In addition, these locally sourced plant materials have fungitoxic potentials in preserving healthy yam tubers in storage, instead of the use of chemical fungicides which pose dangers to humans and crop plants involved. More so, these also have the advantage of being readily available and affordable because they are commonly grown in Nigeria. With regards to the synergistic activity between the plant materials combined, all the combinations showed great synergism against the test organisms. Therefore, due to the emergence of multifungicides-resistant pathogens, control of rot induced pathogens with organic substances combinations, using two or more plant materials is vital and paramount. This is because synergistic interactions can potentially increase efficacy, prevent the emergence of resistance, and provide broader-spectrum of activity than the use of single plant material.

### **RECOMMENDATIONS**

1. Plant extracts combinations have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the control of pathogenic fungal organisms associated with post-harvest spoilage of root and tuber crops.
2. The synergistic effect from the association of various plant materials leads to new choices for the control of post-harvest rot of yam as against the use of synthetic chemical formulations that are expensive and not readily available.

3. The combinations of antifungal agents of plant origin demonstrating *in vitro* synergism against pathogenic agents are most likely to be a means of achieving pragmatic and effective control and prevention of food spoilage, since the development of new classes of antifungal agents is of paramount importance, the crude aqueous and ethanolic extract of *Vernonia amygdalina*, *Azadirachta indica*, *Occimum gratissimum*, *Cymbopogon citratus*, *Carica papaya*, *Citrus sinensis* showed potential synergy on organisms responsible for yam rot, hence they are recommended for the control of rot inducing microbial organisms.

### **CONTRIBUTION TO KNOWLEDGE**

1. There has been dearth of information on the use of combined plant extracts in the prevention of post harvest rot of yam but the inference of this research has provided significant information on the combined effects of plant extracts in the prevention of post harvest rot of yam
2. It was also established that these plant extracts contain different antifungal compounds that can be extracted by companies in the production of fungicides.
3. The findings of this study also revealed that plant extracts were relatively as effective as commercial fungicide (Mancozeb) in the inhibition of rot inducing fungi, hence farmers are advised to use these plant extracts as natural/plant based fungicides because they are eco-friendly, non toxic and readily available.



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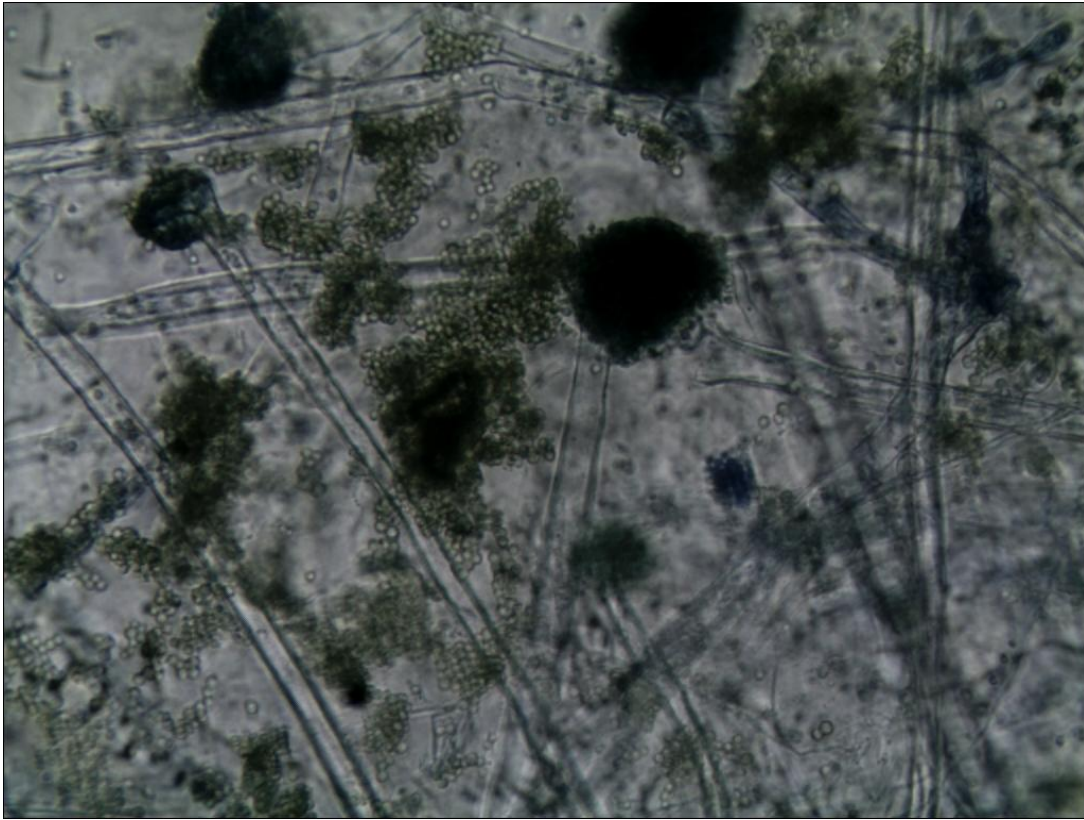
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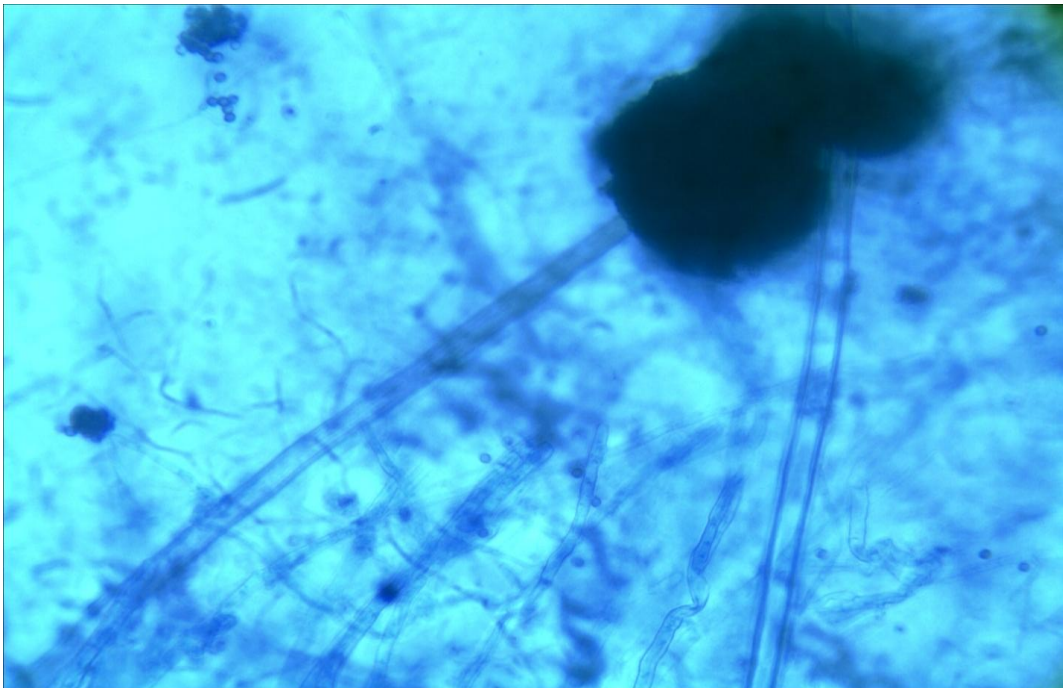
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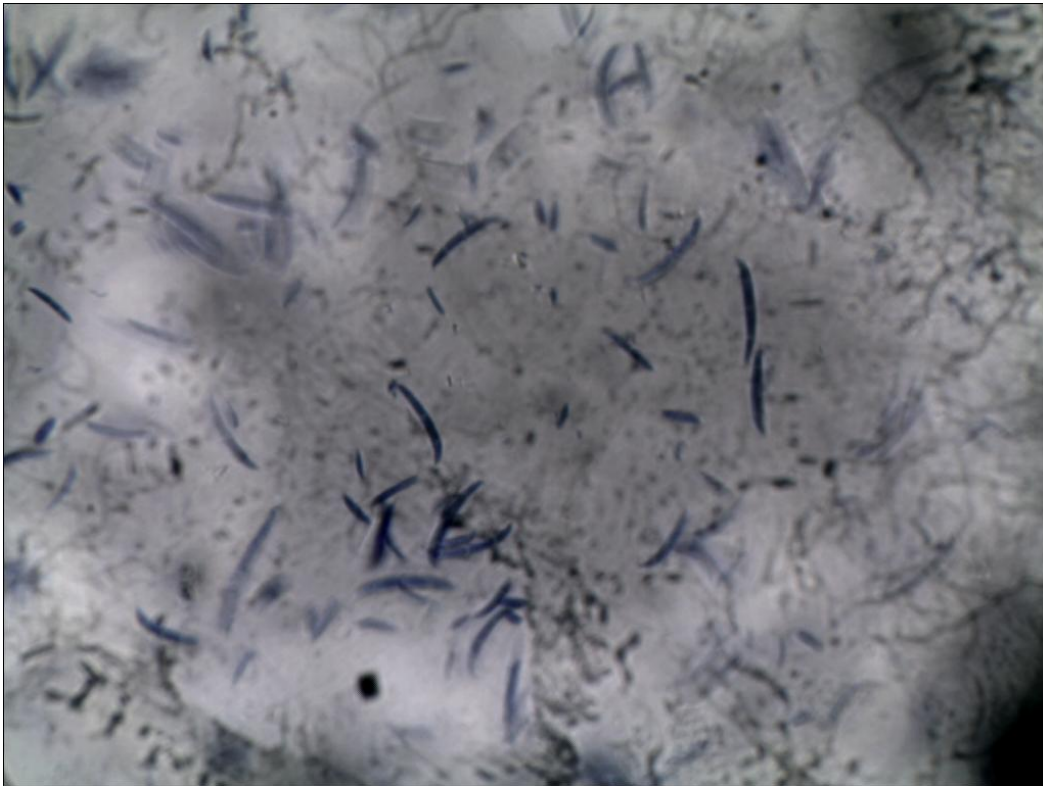
## APPENDIX



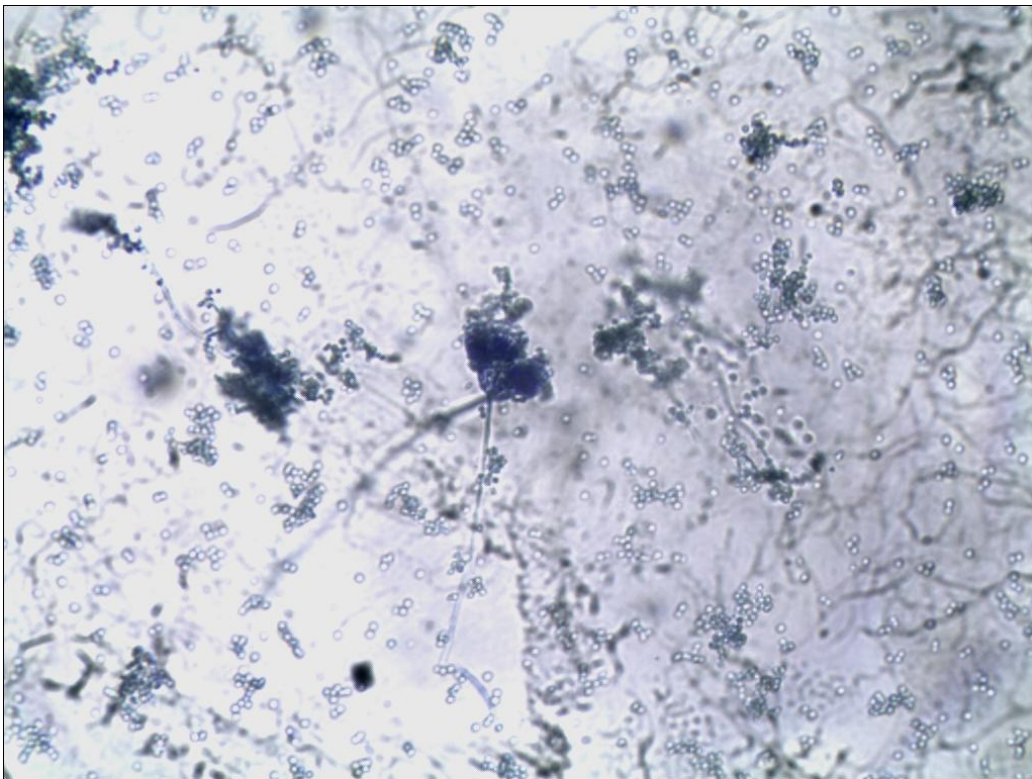
**Plate 7: Photomicrograph of *Aspergillus niger***



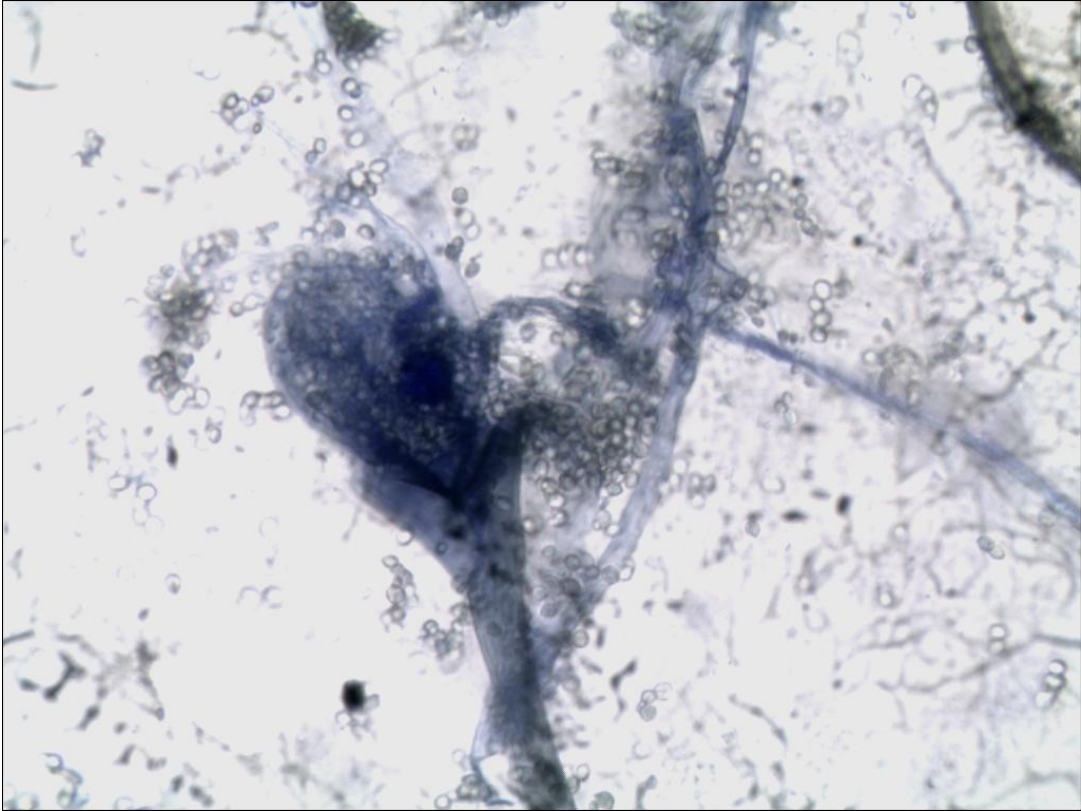
**Plate 8: Photomicrograph of *Aspergillus flavus***



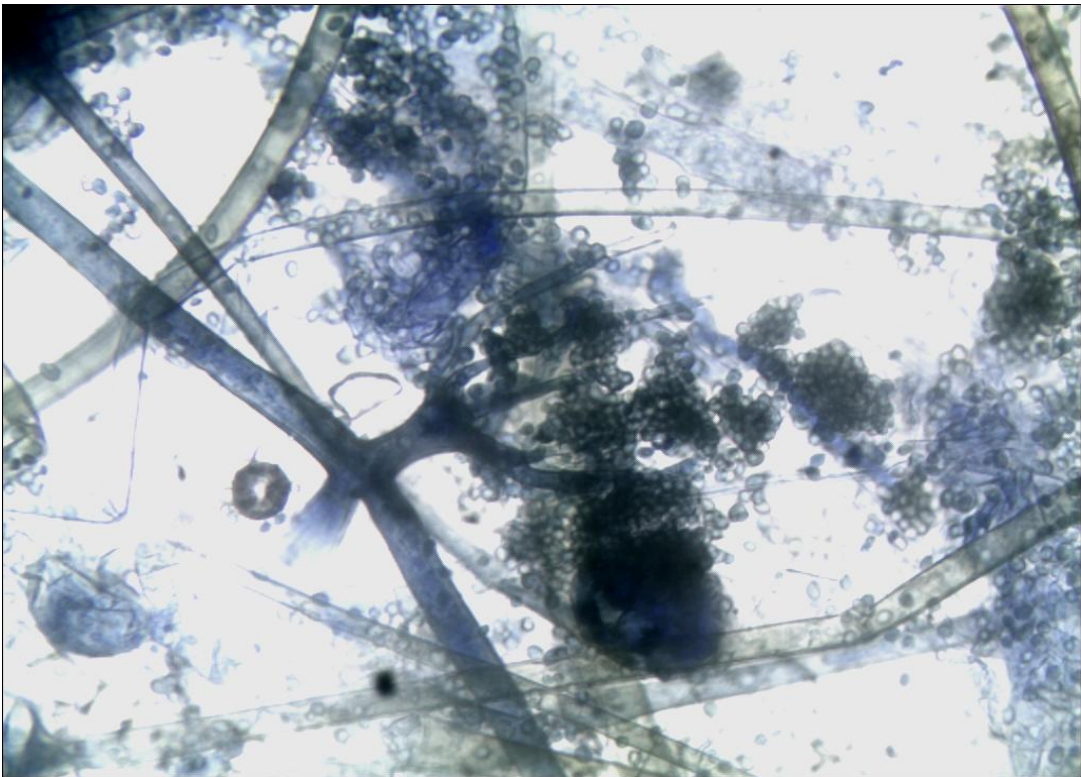
**Plate 9: Photomicrograph of *Fusarium solani***



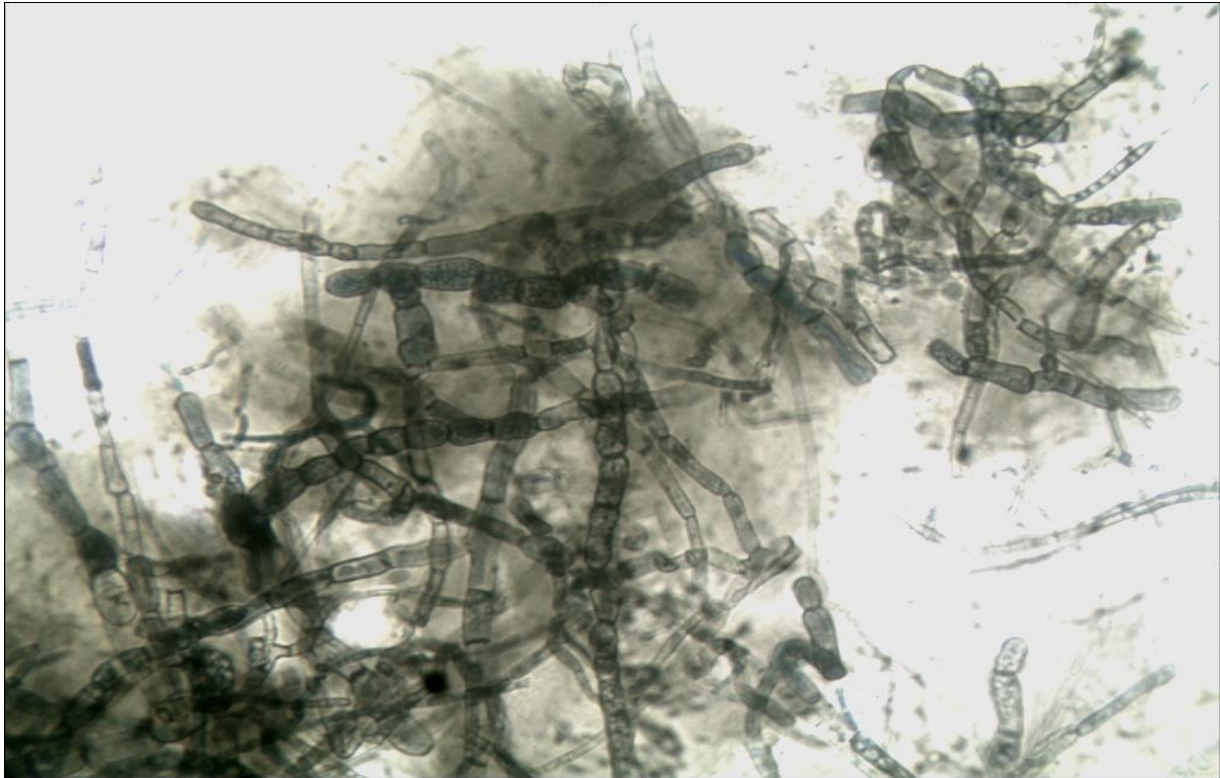
**Plate 10: Photomicrograph of *Penicillium* spp**



**Plate 11: Photomicrograph of *Rhizopus stolonifer* head showing burst**



**Plate 12: Micrograph of *Rhizopus stolonifer* showing rhizoid**



**Plate 13: Photomicrograph of *Botrydiopodia theobromae***



**Plate 14: Healthy yam tubers before inoculation**



**Plate 15: Yam tubers with signs of post-harvest rot, from where fungi organisms were isolated**



**Plate 16: Rotten yam tubers incised through before isolation of fungal organisms**



**Plate 17: Yam tuber inoculated with the spores of the test organisms at various points**



**Plate 18: Incised yam tuber to show extent of rot caused by each inoculated organism**

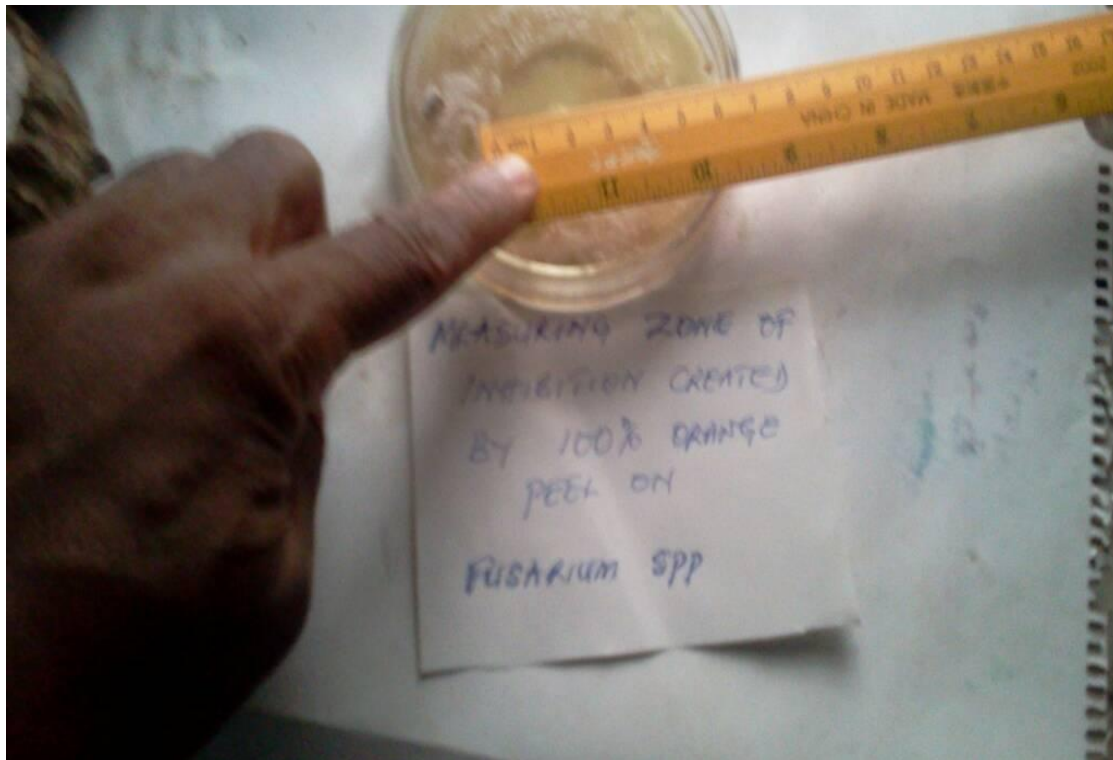


Plate 19: Measuring zone of inhibition created by 10% ethanol extract of *Citrus sinensis* peel on *Fusarium* spp

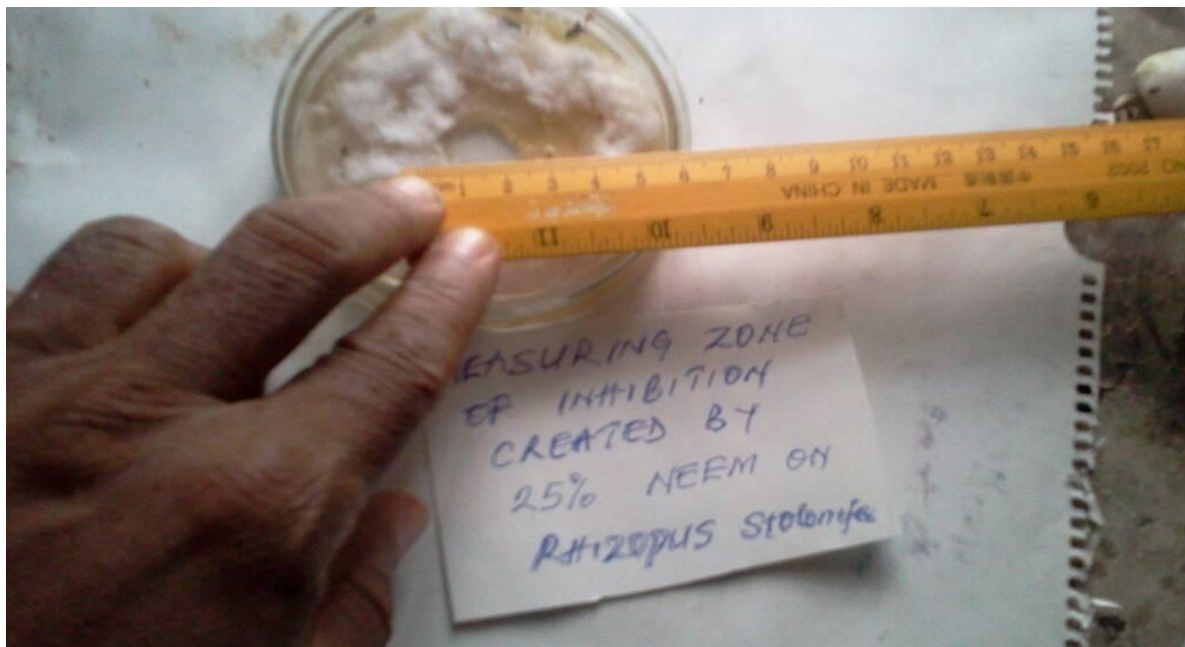


Plate 20: Measuring the zone of inhibition created by 2.5% aqueous extract of *A. indica* on *R. stolonifer*



**Plate 21: Pure cultures of all the organisms isolated from the rotten yam**

**A=***Fusarium solani*

**B=** *Penicillium* spp

**C=** *Aspergillus flavus*

**D=** *Rhizopus stolonifer*

**E=** *Botryodiplodia theobromae*

**F=** *Aspergillus niger*



**Appendix 2: ANOVA for Aqueous Extracts at 2.5 Concentration**

		Sum of Squares	Df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	77877.646	8	9734.706	593.924	.000
	Within Groups	540.886	33	16.390		
	Total	78418.533	41			
<i>RHIZOPUS</i>	Between Groups	15304.177	8	1913.022	2.613	.025
	Within Groups	24158.931	33	732.089		
	Total	39463.108	41			
<i>8BOT</i>	Between Groups	41526.195	8	5190.774	9.630	.000
	Within Groups	17787.504	33	539.015		
	Total	59313.699	41			
<i>PENCILLIUM</i>	Between Groups	51909.759	8	6488.720	60.372	.000
	Within Groups	3546.818	33	107.479		
	Total	55456.577	41			
<i>A.FLAVUS</i>	Between Groups	30137.096	8	3767.137	32.072	.000
	Within Groups	3876.103	33	117.458		
	Total	34013.199	41			
<i>A.NIGER</i>	Between Groups	70237.630	8	8779.704	272.863	.000
	Within Groups	1061.815	33	32.176		
	Total	71299.445	41			

**Appendix 2: ANOVA for Ethanol Extract at 2.5**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	65484.615	8	8185.577	68.296	.000
	Within Groups	3955.224	33	119.855		
	Total	69439.838	41			
<i>RHIZOPUS</i>	Between Groups	18451.305	8	2306.413	2.984	.012
	Within Groups	25503.094	33	772.821		
	Total	43954.399	41			
<i>BOT</i>	Between Groups	41590.637	8	5198.830	9.393	.000
	Within Groups	18263.874	33	553.451		
	Total	59854.511	41			
<i>PENCILLIUM</i>	Between Groups	60882.605	8	7610.326	33.489	.000
	Within Groups	7499.269	33	227.251		
	Total	68381.873	41			
<i>A.FLAVUS</i>	Between Groups	54917.113	8	6864.639	11.281	.000
	Within Groups	20081.539	33	608.531		
	Total	74998.651	41			
<i>A.NIGER</i>	Between Groups	63076.169	8	7884.521	91.651	.000
	Within Groups	2838.927	33	86.028		
	Total	65915.096	41			

**Appendix 3: ANOVA for Aqueous Extracts at 5 Concentration**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	83585.670	8	10448.209	343.373	.000
	Within Groups	1004.128	33	30.428		
	Total	84589.798	41			
<i>RHIZOPUS</i>	Between Groups	15675.753	8	1959.469	2.666	.022
	Within Groups	24255.972	33	735.029		
	Total	39931.725	41			
<i>BOT</i>	Between Groups	41557.549	8	5194.694	9.680	.000
	Within Groups	17708.844	33	536.632		
	Total	59266.393	41			
<i>PENCILLIUM</i>	Between Groups	58341.421	8	7292.678	66.964	.000
	Within Groups	3593.830	33	108.904		
	Total	61935.252	41			
<i>A.FLAVUS</i>	Between Groups	29732.047	8	3716.506	31.900	.000
	Within Groups	3844.682	33	116.506		
	Total	33576.730	41			
<i>A.NIGER</i>	Between Groups	64598.486	8	8074.811	232.665	.000
	Within Groups	1145.289	33	34.706		
	Total	65743.775	41			

**Appendix 4: ANOVA for Ethanol Extracts at 5 Concentration**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	62996.595	8	7874.574	64.252	.000
	Within Groups	4044.395	33	122.557		
	Total	67040.990	41			
<i>RHIZOPUS</i>	Between Groups	20722.532	8	2590.316	3.321	.007
	Within Groups	25739.964	33	779.999		
	Total	46462.496	41			
<i>BOT</i>	Between Groups	44077.470	8	5509.684	9.925	.000
	Within Groups	18318.969	33	555.120		
	Total	62396.439	41			
<i>PENCILLIUM</i>	Between Groups	63934.408	8	7991.801	35.942	.000
	Within Groups	7337.609	33	222.352		
	Total	71272.016	41			
<i>A.FLAVUS</i>	Between Groups	69307.656	8	8663.457	24.863	.000
	Within Groups	11498.694	33	348.445		
	Total	80806.350	41			
<i>A.NIGER</i>	Between Groups	71051.088	8	8881.386	110.786	.000
	Within Groups	2645.517	33	80.167		
	Total	73696.605	41			

**Appendix 5: ANOVA for Aqueous Extracts at 7.5 Concentration**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	85090.587	8	10636.323	234.082	.000
	Within Groups	1499.472	33	45.439		
	Total	86590.059	41			
<i>RHIZOPUS</i>	Between Groups	16105.599	8	2013.200	2.637	.024
	Within Groups	24433.856	32	763.558		
	Total	40539.455	40			
<i>BOT</i>	Between Groups	42409.731	8	5301.216	9.879	.000
	Within Groups	17708.754	33	536.629		
	Total	60118.486	41			
<i>PENCILLIUM</i>	Between Groups	70387.814	8	8798.477	80.980	.000
	Within Groups	3585.458	33	108.650		
	Total	73973.272	41			
<i>A.FLAVUS</i>	Between Groups	35790.889	8	4473.861	38.489	.000
	Within Groups	3835.800	33	116.236		
	Total	39626.688	41			
<i>A.NIGER</i>	Between Groups	68320.660	8	8540.083	307.781	.000
	Within Groups	915.661	33	27.747		
	Total	69236.321	41			

**Appendix 6: ANOVA for Ethanol Extracts at 7.5 Concentration**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	67482.035	8	8435.254	72.572	.000
	Within Groups	3835.670	33	116.232		
	Total	71317.705	41			
<i>RHIZOPUS</i>	Between Groups	39614.067	8	4951.758	6.389	.000
	Within Groups	25575.424	33	775.013		
	Total	65189.491	41			
<i>BOT</i>	Between Groups	59704.515	8	7463.064	13.691	.000
	Within Groups	17988.619	33	545.110		
	Total	77693.133	41			
<i>PENCILLIUM</i>	Between Groups	64421.706	8	8052.713	35.305	.000
	Within Groups	7526.959	33	228.090		
	Total	71948.665	41			
<i>A.FLAVUS</i>	Between Groups	68940.680	8	8617.585	24.733	.000
	Within Groups	11498.019	33	348.425		
	Total	80438.699	41			
<i>A.NIGER</i>	Between Groups	75743.589	8	9467.949	137.053	.000
	Within Groups	2279.722	33	69.082		
	Total	78023.311	41			

**Appendix 7: ANOVA for Aqueous Extracts at 10 Concentration**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	83903.364	8	10487.921	740.928	.000
	Within Groups	467.119	33	14.155		
	Total	84370.483	41			
<i>RHIZOPUS</i>	Between Groups	16223.767	8	2027.971	2.760	.019
	Within Groups	24246.314	33	734.737		
	Total	40470.081	41			
<i>BOT</i>	Between Groups	45596.602	8	5699.575	10.619	.000
	Within Groups	17712.754	33	536.750		
	Total	63309.356	41			
<i>PENCILLIUM</i>	Between Groups	70605.028	8	8825.629	79.926	.000
	Within Groups	3643.950	33	110.423		
	Total	74248.979	41			
<i>A.FLAVUS</i>	Between Groups	56288.075	8	7036.009	62.483	.000
	Within Groups	3716.032	33	112.607		
	Total	60004.107	41			
<i>A.NIGER</i>	Between Groups	75064.402	8	9383.050	326.680	.000
	Within Groups	947.840	33	28.722		
	Total	76012.242	41			

**Appendix 8: ANOVA for Ethanol Extracts at 10 Concentration**

		Sum of Squares	df	Mean Square	F	Sig.
<i>FUSARIUM</i>	Between Groups	67940.125	8	8492.516	63.429	.000
	Within Groups	4418.405	33	133.891		
	Total	72358.530	41			
<i>RHIZOPUS</i>	Between Groups	46226.383	8	5778.298	7.442	.000
	Within Groups	25623.998	33	776.485		
	Total	71850.380	41			
<i>BOT</i>	Between Groups	59308.718	8	7413.590	13.518	.000
	Within Groups	18098.559	33	548.441		
	Total	77407.276	41			
<i>PENCILLIUM</i>	Between Groups	65598.708	8	8199.838	36.733	.000
	Within Groups	7366.469	33	223.226		
	Total	72965.176	41			
<i>A.FLAVUS</i>	Between Groups	66909.946	8	8363.743	26.790	.000
	Within Groups	10302.344	33	312.192		
	Total	77212.290	41			
<i>A.NIGER</i>	Between Groups	77298.388	8	9662.299	140.363	.000
	Within Groups	2271.647	33	68.838		
	Total	79570.035	41			