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Upgrading Antifungal Properties of Cultivated Basil (*Ocimum gratissimum* L.) Through Soil Amendments

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Abstract
Fungal infections have been on the rise, prompting a search for alternative control methods due to concerns about resistance, environmental persistence, and adverse effects of synthetic fungicides on non-target organisms. Basil (*Ocimum gratissimum*) has been recognized for its antifungal properties and widespread usage. Meeting the high demand for basil necessitates commercial production, typically requiring soil amendments. However, the specific soil amendment best suited to enhance basil's antifungal properties remains unknown. This study aims to identify the type of soil amendment for cultivating basil with heightened fungicidal efficacy. Poultry, cattle, and goat manure, along with NPK fertilizer, were utilized as soil amendments for a four-month basil cultivation experiment. Beds measuring 1m x 2m were allocated for each treatment in a randomized complete block design, with five treatments and three replicates. Basil leaf extracts obtained from each soil amendment type were serially diluted to varying concentrations, combined with molten agar, and poured into separate Petri dishes. Fungal inoculum was then introduced to assess the inhibitory effects of basil extracts. The minimum inhibitory concentration of the extracts was determined as the lowest concentration of extracts which inhibit the visible growth of the fungi. Results indicated that poultry manure was the most effective soil amendment for enhancing basil's antifungal capacity.

Keywords: *Ocimum Gratissimum,* Cattle Manure, Goat Manure, Poultry Manure, NPK Fertilizer, Fungicides.

Introduction

Fungi are the most prominent causes of diseases (Jimoh et al., 2020) as they have been implicated in many diseases in man and other animals, these diseases include candidiasis, dermatitis, eczema, scabies, thrush and foot and mouth diseases. It is noteworthy that more than 80% of crop diseases are reported to have been caused by fungi. Notable fungi pathogens of crops include *Aspergillus spp, Fusarium spp, Botrytis spp, Alternaria spp, Phytophthora spp, Xanthomonas spp, Penicillium spp* and *Pseudomonas spp*. These fungi attack crops like cereals, legumes, fruits and vegetables, tubers and tree crops. Examples of fungal diseases are smut, blight, black pod, damping off stem and root rot, wilts and leaf spots. Fungicides are chemical substances used to destroy fungi or their spores, they include thiram, perenox, captan, Bordeaux mixture and copper sulphate miconazole, terbinafine, amorolfin, amphotericin and benomyl. In recent times, despite the progress made in modern medicine, there is an increase in fungal infection. There is therefore the need to search for effective antifungal agents to control fungal diseases. The problems of resistance and persistence to the environment and other side effects of synthetic fungicides to non-target organisms call for a search for better control agents (Maimone et al., 2021). Due to the undesirable effects of most of the available synthetic fungicides such as high and acute toxicity, low degradation period and accumulation in the food chain and undesirable extension of their power to destroy useful microorganisms; there are restrictions against their use (Satish et al., 2009). Besides, synthetic fungicides are known to cause carcinogenicity, and teratogenicity on non-target organisms as well as pollution of the environment (Satish et al., 2009). Hence, there is a serious search for effective and safer fungicides to control animal and crop fungal diseases.

Plants have been described as the sleeping giants which possess the bioactive agents that can solve the problem of antibiotic resistance which is currently a major global issue. Several researchers have reported the antifungal activities of some plants (Satish et al., 2009; Siva et al 2008; Abdallah et al., 2011). These plants include *Azadirachta indica, Mallotus oppositifolius, Funtumia elastic, Senna alata, Vernonia tenoreana, Acalypha fimbriate, Carica papaya, Corchorus Olitorius Hibiscus sabdariffa, Coconus nucifera, Chrysanthemum Americana, Terminalia avicerinoides, Agerantum conyzoides* and *Ocimum gratissimum* (Aina et al., 2019). Farombi (2013) also affirmed that higher plants possess bioactive substances which are active against fungi and could be used in disease control. According to Amadi et al. (2010), basil (*Ocimum gratissimum*) possessed antifungal activities against *Aspergillus repens, Curvularia lunata* and *Fusarium moniliforme.* Basil leaves have also been reported to inhibit the growth of *staphylococcus spp, Enterococcus spp,* and *Aspergillus spp* (Tanackov et al., 2011). Several researchers have shown that the leaves of basil exhibit antifungal activities against *Fusarium moniliforme, Aspergillus fumigatus Aspergillus flavus, Penicillium spp., Botrytis spp.* Abdallah et al. (2011), Viera et al. (2014), however, specified that the antifungal compounds in basil are estragol, linoliol, camphor and eugenol.

Ocimum gratissimum – Basil is a shrub which belongs to the family Lamiaceae. The plant is rich in aromatic compounds and essential oils. It is found in the tropical and sub-tropical regions of the world, particularly in India and Nigeria. In Nigeria, basil is called Dandoya in Hausa, Nchu anwu in Igbo and Efinrin in Yoruba. Basil has been used in preparing culinary dishes, traditional medicine, crop protection and food preservatives (Tagne, 2000). The plant helps in skin health in humans, boosting digestion and detoxifying body systems. The active ingredients in basil leaf extracts have been shown to include tannins, saponnins, flavonoids, fatty acid, phenol, riboflavin, thyamin, polyphenols, rosmarinic acid, catechin, eugenol and epicatechin. The plant has been revealed to contain many active ingredients such as flavonoid, triterpenes, alkaloids, citrasapon, eugenol, linaol, methyl-cinnamate, camphor and thymol (Okpala, 2015). The extract is also a good source of minerals such as Ca, K, Mg, Na, Fe, Zn, Vit A and Vit k. Leaves of basil have been found to exhibit antifungal activities against fungi which include *Fusarium moniliforme, Aspergillus fumigatus* and *Aspergillus flavus, Penicillium spp., Botrytis spp*. Basil leaves have been reported to inhibit fungal pathogen growth such as *staphylococcus spp*, *Enterococcus spp* and *Aspergillus spp* (Tanackov et al., 2011). Siva et al. (2008) stated that the compounds in basil are biodegradable and also selectively toxic, hence of value in disease control. Approximately 25% of the active substance prescriptions in the United States of America came from plant materials (Cespedes et al., 2006). Basil is a thermophilic plant from the tropics which grows and develops optimally only if the temperatures are high enough during the entire growing period (Majkowska-Gadomska et al., 2017). Nigeria's climatic conditions are favourable for basil production. Basil can be fully explored by cultivating it in large quantities to use in traditional medicine, preparation of culinary dishes and fungal drug development. The use of appropriate soil amendments enhances the secondary metabolites in plants and thus improves their fungicidal activities (Aina et al., 2019; Ibrahim et al., 2013).

Most of the lands used for crop production in Nigeria require the application of soil amendments because of the continuous use of land for crop production. Soil amendments are materials added to the soil to improve the nutrient and physical properties of the soil. They are organic fertilizers such as compost, cattle manure, goat manure and poultry manure and inorganic fertilizers such as NPK, urea, lime and sulphur. It is noteworthy that bioactive substances in medicinal plants are the target materials for their pesticidal efficacies. Aina et al. (2019) reported that the use of inorganic fertilizers increases the bioactive substances in tomatoes while Ibrahim et al. (2013) affirmed that organic fertilizer increases bioactive substances in *Lavinia pumila*. This means that medicinal crops require different types of soil amendments to boost their efficacies. Therefore, the right type of soil amendment must be chosen for the cultivation of basil to get the desired secondary metabolites for effective antifungal properties. Hence, it is a necessity to know the actual type of materials required to be added to soil to improve the anti-fungal properties of growing basil. This study aimed to evaluate the effects of cattle, goat and poultry manure and inorganic (NPK) soil amendments on the fungicidal properties of basil. Several researches have been conducted on fungi as well as basil, but there is little or no information on the appropriate soil amendment required by basil for higher bioactive compounds and thus, higher fungicidal capacity. Research on large-scale cultivation of basil is scanty, but this investigation can fetch this crop (basil) more attention to boost its production for human use. The demand for basil has risen significantly for its diverse applications, yet its cultivation remains largely confined to home gardens. There is a lack of understanding regarding how various soil amendments affect the fungicidal potentials of basil. Since different soil amendments can influence the levels of bioactive compounds in plants, determining the most suitable type of soil amendment for cultivating basil to maximize its antifungal properties remains a gap in current knowledge. The objectives of this study were to i. Evaluate the influence of NPK fertilizer on the antifungal properties of basil; ii. Explore the impact of cattle manure on the antifungal activities of basil; iii. Investigate the effects of cattle manure on the antifungal properties of basil and iv. Assess the effects of poultry manure on the antifungal activities of basil.

Materials and Methods

The experiment was conducted between March and November 2023, at the experimental farm of the Department of Agricultural Education, Federal College of Education (Sp), Oyo, Oyo State, Nigeria. Farmland which has been used for vegetable cultivation for five years without any soil amendment was used for the cultivation of basil. Chicken droppings and goat and cattle dung were collected daily for two weeks from their respective pens at the Department of Agricultural Education farm, along with their bedding materials. These materials were packed separately in shaded heaps and supplemented with domestic wastes. The heaps were left to decompose for two weeks, turned weekly, and then utilized for basil cultivation. Beds measuring 1m x 2m were utilized for the experiment. Each block comprised five beds, with each bed representing a specific treatment. The experimental design followed a randomized complete block (RCBD) layout, with five treatments and three replicates, totalling 15 beds. The treatments included:

T1: Treatment with cattle manure
T2: Treatment with goat manure
T3: Treatment with poultry manure
T4: Treatment with NPK fertilizer
T5: Control group with no soil amendment

Seeds of basil were sown and nurtured in the nursery for five weeks. Beds designated to receive poultry, cattle, and goat manure were added accordingly and thoroughly mixed with soil at a rate of 10 tons per hectare. After watering, the beds were left to mineralize for a week. Following this, five-week-old seedlings were transplanted onto all beds with a spacing of 30cm within and between rows. Two weeks later, NPK fertilizer was applied to the corresponding beds at a rate of 100 kg per hectare. The plants were tended to until 90 days after seeding when harvesting took place. The leaves were harvested and utilized for the investigation. Subsequently, the harvested leaves were oven-dried at 40°C until reaching a constant weight and then ground into a powdery form. Extraction was carried out using ethanol and water. Approximately 50g of each pulverized plant sample was shaken at 120 rpm for 24 hours in a mechanical shaker. The resulting extracts were filtered using Whatman No.1 filter paper connected to a Buchner funnel attached to a vacuum pump. The filtrate was then concentrated to dryness using a rotary evaporator set at 78°C. Aqueous filtrates were chilled at -40°C and further concentrated to dryness within 24 hours using a freeze dryer. The resulting extracts were reconstituted in distilled water to obtain a stock solution of 200 $mgml^{-1}$. Serial dilutions of the sample extracts were prepared (100, 50, 25, and 12.5 $mgml^{-1}$) in the broth. Five fungi were examined in this study: *Aspergillus flavus, Candida albican, Fusarium oxysporum., Penicillium aurantiogriseum* and *Ustilago maydis*

A fungi spore density of about 10^5 spores/ml was prepared using a spectrophotometer at 580 nm. The method described by Jimoh et al. (2020) was used in preparing this assay. Sabourand agar was prepared for this investigation. Agar of 19 ml was put in the Erlenmeyer flasks and autoclaved at 121 ^oC for 15 min, this was then allowed to stand at 55 ^oC in the water bath. One millilitre of each of the serially diluted extracts was mixed with the 19 ml molten agar to give a concentration range of 10 $mgml^{-1}$ (highest concentration) to 0.625 $mgml^{-1}$ (lowest concentration). The mixture of agar and plant extracts was poured into each of the Petri dishes which were already divided into sections to accommodate each fungus. These were left to cool and solidify. Then, 10 µl of fungal inoculum was put on the solidified agar at the corresponding section of the Petri dish to give the desired final inoculum of $1x10^4$ CFU/ spot. Benomyl, a systemic fungicide, was used as a guide for fungal growth at the concentration range of 10 μgml^{-1}. The preparation was incubated at 30 ^oC for 72 h. Thereafter, the Petri dishes were checked for fungal growth.

A fungal spore density of approximately 10^5 spores/ml was prepared using a spectrophotometer at 580 nm. The method described by Jimoh et al. (2020) was followed for this assay. Sabouraud agar was prepared, and 19 ml of agar was autoclaved in Erlenmeyer flasks for 15 minutes at 121°C. After cooling to 55°C, 1 ml of each serially diluted extract was mixed with the molten agar to achieve a concentration range of 10 $mgml^{-1}$ (highest) to 0.625 mg ml^{-1} (lowest). The mixture was poured into Petri dishes divided into sections to accommodate each fungus and left to

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solidify. Subsequently, 10 µl of fungal inoculum was placed on the solidified agar in the corresponding section of the Petri dish to attain the desired final inoculum of $1x10^4$ CFU/spot. Benomyl, a systemic fungicide, was used as a guide for fungal growth at a concentration range of 10 $µgml^{-1}$. The prepared plates were then incubated at 30°C for 72 hours, after which fungal growth was assessed. The minimum inhibitory concentration of extracts was determined as the lowest concentration of extracts which inhibit the visible growth of the fungi.

Results

Table 1: Minimum inhibitory concentrations of different basil extracts on fungi

	Aspergillus flavus $mgml^{-1}$		*Candida albicans* $mgml^{-1}$		*Fusarium oxysporum* $mgml^{-1}$		*Penicillium aurantiogriseum* $mgml^{-1}$		*Ustilago maydis* $mgml^{-1}$	
Treatment	Aqu	Eth	Aqu	Eth	Aqu	Eth	Aqu	Eth	Aqu	Eth
Cattle manure	5.0	2.5	5.0	2.5	1.25	1.25	2.5	2.5	1.25	1.25
Goat manure	2.5	2.5	2.5	2.5	1.25	1.25	5.0	5.0	1.25	1.25
Poultry manure	<0.625	<0.625	1.25	<0.625	<0.625	<0.625	1.25	1.25	1.25	1.25
NPK Fertilizer	2.5	1.25	2.5	1.25	2.5	2.5	<0.625	<0.625	2.5	2.5
Control	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10

Eth: ethanolic extract, Aqu: Aqueous extracts

Effects of cattle manure on the tested fungi: Aqueous extracts of basil treated with cattle manure partially (MIC 5.0 mg ml^{-1}) inhibited *Aspergillus flavus* and *Candida albicans* while other aqueous extracts and all ethanolic extracts highly inhibited the growth of all the tested fungi (MIC1.25, 2.5 $mgml^{-1}$). This shows that cattle manure is good for cultivating basil intended to be used for controlling these fungi, however, an aqueous extract of basil treated with cattle manure may not be good for growing basil for controlling *Aspergillus flavus* and *Candida albicans.* A previous study showed that cattle manure has a strong suppressive effect on the soil pathogenic fungal growth and may regulate antagonistic groups (Sun et al., 2016). This reveals that cattle manure contains some fungi-inhibiting compounds which may have been imbibed by basil and raises its fungicidal capacity, however, water may not be the suitable medium for extraction of basil active ingredients in controlling some fungi.

Effects of goat manure on the tested fungi: Both aqueous and ethanolic extracts of basil treated with goat manure inhibited (MIC1.25, 2.5 mg ml^{-1}) all the tested fungi except *Penicillium aurantiogriseum* whose growth was partially inhibited in both media (MIC 5.0 mg/mL).In this study, goat manure may not be suitable as soil amendment to grow basil intended for controlling *P aurantiogriseum* while the manure can be used to grow basil for controlling other fungi. Guo et al. (2022) reported that goat manure can inhibit the growth of some fungi and is less active against some *P.aurantiogriseum* is likely to be one of them.

Effects of poultry manure on the tested fungi: Both aqueous and ethanolic extracts of poultry manure highly inhibited the growth of all the fungi. *Aspergillus flavus, and Fusarium oxysporum* were perfectly suppressed (MIC <0.625 mg ml^{-1}) in both media while *Candida albicans* were perfectly suppressed in ethanolic medium. This shows that the fungi are very susceptible to poultry manure-treated basil. Poultry manure may have contained some active ingredients which reacted with basil substances and produced bioactive substances that worked highly against fungi especially *Aspergillus flavus, Fusarium oxysporum* and *Candida albicans.* This study reassures the report of Guo et al. (2022) that manure reduces fungi diversity, the fungi used in this work may have been among all the fungi poultry manure can suppress. The antifungal substances present in the poultry manure may have promoted the activities of basil extracts against all the tested fungi. It was reported that leaf extracts of basil completely inhibited the growth of *Fusarium spp* and *Aspergillus flavus* (Dambolena et al., 2010).

Effects of NPK fertilizer on the tested fungi: NPK extracts inhibited the growth of all the fungi in various capacities (2.5, 1.25, <0.625 mic). In this study, NPK showed higher potency (MIC<0.625 mg ml^{-1}) against *P. aurantiogriseum* than other treatments. This confirms the report of Aina et al. (2019) that mineral fertilizer has higher secondary metabolites which improve plant fungicidal capacity, but the result was not in line with the report of Ibrahim et al. (2013) and Di Renzo et al. (2007) who stated that organically produced foods have higher antioxidant activities due to presence of higher phytochemical contents.

Control

Treatment without any soil amendment (negative control), could not inhibit the growth (MIC >10 mg ml^{-1}) of any of the treated fungi as mycelia masses were seen on the petri dishes of control. Benomyl (positive control) inhibited the growth of all the organisms as no traces of mycelial mass were seen. Table 1. The result obtained showed that basil has antifungal capacity against all the tested fungi, although there were differences in the levels of inhibitory capabilities of the treatments. *Ocimum gratissimum* has been revealed to contain many active ingredients such as flavonoid, triterpens, alkaloids, citrasapon, eugenol, linaliol, methyl-cinnamite, camphor and thymol (Okpala, 2015). Abdullah et al., 2011); Viera et al.,2014), however, specified that the antifungal compounds in basil are estragol, linoliol, camphor and eugenol. This supports the report of Marco et al., (2020) which affirms the anti-fungal effects of basil. The result from this study shows that all the treatments inhibited the growth of the fungi at different ranges. Among all the treatments, poultry manure is the most suitable to use as a soil amendment for basil because of its higher efficacy in fungal control. Although NPK performed somewhat better against *P. aurantiogriseum*, poultry manure treatment worked well against all the fungi treated. Besides, poultry manure is environmentally benign while NPK leads to environmental degradation. Han et al. (2016) stated that the use of mineral fertilizer results in problems such as nutrient loss, soil acidification, soil water contamination and reduction in useful soil microorganisms. Also, Salehi (2019) reported that the use of organic fertilizer alone significantly promoted the antioxidant activity, and total phenolic content of harvested seeds compared to chemical fertilizer. Cattle and goat manure did not work against fungi as much as poultry manure, nevertheless, they can also be used as a soil amendment for basil cultivation especially when poultry manure is not available. Lundegardhi and Martensson (2003) reported that organically produced plant foods contain higher amounts of secondary compound metabolites which may be more health-promoting than those produced through conventional means. Salehi et al (2017); Costa et al. (2013) stated that to ensure high content of bioactive compounds in the plants, sufficient nutrients must be available in the soil for the plant, from this research, the use of organic fertilizer, particularly poultry, proved to be the best for basil cultivation.

Conclusion

Poultry manure-treated soil was outstanding in inhibiting all the fungi compared to others, and even better than NPK treatment, thus, poultry manure should be used as soil amendment to cultivate basil for higher antifungal efficacy. Cattle and goat manure may also be used if poultry manure is not available.

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