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Biological Activities and Phytochemical Constituents in the Stimulatory Potential of *Tithonia diversifolia* Fermented Extracts: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The world food production must increase by 70% to feed this massive population through the use of chemical inputs in the new millennium. Crop culture is challenged by parasitic constraints, seedlings unavailability and soil fertility issues, which can severely reduce productivity. However, the repeated uses of chemical inputs negatively impact environment, plant and human health equilibrium. So, pathogen resistance, seedling quality and plant fertilization are severely concerned over the course of time. In this context, the use of a plant extract as *Tithonia diversifolia*, could be a good natural alternative to chemical inputs stimulants. The lack of knowledge of phytochemical constituents involving biologicals activities in liquid form of *T. diversifolia* extract, need to be improved to ensure their labeling. This study reviews biological activities and main phytochemical constituents in the stimulatory potential of *T. diversifolia* fermented liquid extracts. The stimulatory potential bio-efficacy of *T. diversifolia* extracts concerns the combination of biocontrol and green fertilizer effects. The green manure is conducted by watering or mulching on varied crops, such as rice, maize or plantain banana. Existing concerns are to expand the information amounts concerning the stimulating properties of the bioactive compounds contained in the fermented liquid extracts. It would be a key to fully demonstrate their effectiveness in stimulating the mechanism of plant growth and the protection against phytopathogens. However, this review updates on the challenge of the possibility to characterize the major bioactive compounds in the extracts. The other challenge of this current work is to suggest an eco-responsible and biological labeling product based on *T. diversifolia* fermented liquid extract.

Keywords: T. diversifolia; fermented extract; biological activity; phytochemical constituent; stimulatory.

1. INTRODUCTION

The fundamental preoccupation of the Food and Agricultural Organization and United Nation is to feed the increasing world population. This population is estimated to reach around 9.6 billion in 2050, with no corruptible and unpolluted nutrients. Therefore, world food production must increase by 70% to feed this massive population [1]. However, some agricultural constraints remain, preventing this objective to being achieved. In agriculture, pathogens attacks and seeds qualities are the main constraints causing production reductions. They are in constant competition with crops for water, light and nutrients resources coupled sometimes with soil fertility, which lead to huge economic losses. Among the physical or mechanical control methods, including stripping or cutting, chemical inputs such as pesticides, insecticides or fertilizers are the most used ones. However, it is well known that their repetitive and intensive uses, impact negatively human health, plant and environment equilibrium as well as increasing pesticide resistances. The use of phytopharmaceuticals is yet more rigorous, with more than 90% of the cost of the study conferred to approval expenses against a few for efficiency agronomic analysis approach [2]. Therefore, the number of active products implied the development of alternative methods is decreasing. The plant protection strategies could increase the food production while greatly reducing the environmental impacts of agriculture. One of these strategies relies on the optimization of natural defense mechanisms of plants, set up during plant-parasite interactions [3]. In this context, natural products based on *T. diversifolia* liquid extracts containing several bioactive compounds, could be a real alternative to phytopathogen attacks and soil fertility issues [4-6].

T. diversifolia is a woody herb of 2-3 meters tall which is native to East of Mexican country and Central America. The species is spreading quickly and has naturalized in more than 70 countries in tropical areas, where it is an aggressive invader with 80,000 to 160,000 seeds produces annually and germination rates ranging from 18 to 56% in the new area (Chagas et al., 2012). The plant is rich in elements such as nitrogen (N), phosphorus (P) and potassium (K). Its applications on soils result to rapid decompositions. Thereby, it contributes to the growth promotion of plants, by enriching the soil with N, P and K [7,8]. *T. diversifolia* extract is rich in following secondary metabolites: flavonoids, tannins, alkaloids, pathogenesis-related proteins and terpenoids. This chemical composition seems to stimulate the plant growth and its accumulation of defense biomarkers. The plant is known to be a good source of bioactive compounds and to have therapeutic potential with antifungal, antimicrobial, insecticidal and organic fertilizing (green manure) properties. These characteristics reinforce the importance of developing a product based on biostimulant potentials [9,10]. The stimulatory potential of *T. diversifolia* extracts and their compounds can be isolated from new forms as concentrated fermented liquid, mulch, and powder and especially studied in some plant models as plantain banana, rice, and maize. This current study on fermented liquid showed a large spectrum of their biological's activities.

Recent studies on plantain banana seedlings "Plants Issus de Fragments de tiges: PIFs" in Cameroon have shown that *T. diversifolia* extracts, used alone or associated with clam shells, are profitable for plants. Indeed, it stimulates the plant growth and the protection against biotic and abiotic stresses, with watering, mulching or amendment applications [11,12,6]. Moreover, plantain banana is an important cash crop that serves as a stable food for millions of people in the world and contributes to coming generations. Nevertheless, this production encounters many problems, concerning parasitic constraints, availability of good seedlings in quantity and quality, and soil fertility which have caused the lack of creation of new plantations. All these points increase the demand and generate very high prices with an average of 300 to 500% on the local market [13]. In the fields, *Mycosphaerella fijiensis* fungus is known to attack specially plantain banana, which is one of the main concerns target pathogen in its cultivation. Indeed, the strain can severely reduce the photosynthetic leaf area, leading to losses of plantain banana production beyond 80%. Soil fertility constitutes another main preoccupation, because their combination with black sigatoka disease can totally reduce plantain banana productivity. Many studies cases in the fields and the greenhouse concern plantain banana, *T. diversifolia* extracts increased the plant growth, plant protect and the productivity of other plants. Here will be presented the relationship between phytochemical constituents contained in *T. diversifolia* extracts and some biological activities in stimulatory potential on plantain banana. The challenge of this study is to put forward an eco-responsible and biological labeling product based on a *T. diversifolia* fermented liquid extract, with the knowledge of its major bioactive constituents.

2. METHODS

Many studies on *T. diversifolia* extracts published were obtained from biopesticidals and biofertilizers efficiency treatment databases. Some publishers or data base such as Google Scholar, Science Direct, African Journal of Botany, Agricultural Sciences, American Chemical and Agriculture Society, Plant Pathology, Science Direct, Royal Society of Chemistry, Scopus and SciFinder permitted to collect the right information for understanding the stimulatory potential of extracts and the biologicals activities of phytochemicals constituents. The keywords used were *T. diversifolia*, fermented extract, biostimulant, biological activities, and phytochemical constituent. In addition, words like extraction of volatile and non-volatile components were also used during the search.

3. RESULTS AND DISCUSSION

3.1 Biological Activities of *T. diversifolia*

3.1.1 Fertilizer

T. diversifolia plant cut from bushes or harvested in cultivated areas, are incorporated into subtropical crop fields as sources of nutrients in dried, ash or liquid forms. It is as green manure that can bring positive modifications in soils. It increases N, P and K levels compared to an inorganic fertilizer or other plants such as *Tephrosia* or maize stover. *T. diversifolia* biomass can be decomposed rapidly to release N, P and K into the soil. This is the reason why this biomass has been extensively used to improve soil fertility [14]. The shoot biomass of *T. diversifolia* is hawked as a potential source of nutrients for lowland rice in Asia and more significantly for maize and vegetables. During the first season of maturing, the application of 3 to 4.5 mg of *T. diversifolia* fresh extract of leaves gives similar yield rates to those with 90 mg of N as inorganic fertilizer. *T. diversifolia* fresh extract increases significantly maize yields, when it is available or growing naturally on fields, and properly used. *T. diversifolia* is assumed to be rich in nutrients, so leaves are used as green manure on poorly fertile soils [15]. Therefore, the soil is improved through N and P release, resulting in an enhancement from the first maize (*Zea mays*) season in yield and quality [16]. Similarly, physicochemical and biological properties as fertilizer or green manure conferred to *T. diversifolia* turn it into an excellent supplier in N, P and K elements. The high rate of its quickly decomposition also enrich poor fertile surrounding soils in fertilizer substances including mineral elements [17,18].

3.1.2 Pesticidal

T. diversifolia is widely used to control a lot of phytopathogens or plants diseases. The extract efficiency depends on the choice of the tree section and the extraction solvent. The various processes and solvents used before obtaining an extract make possible to show this potentiality. However, cold infusion of the leaves, stems, roots or the whole mixture of *T. diversifolia* has been shown to be effective as a pesticide against aphids, weevils and beetles [19] on common bean plants [20]. Crushed and ethanolic extracts of *T. diversifolia* leaves, applied at concentrations between 2% to 5% on cowpea, allowed a mortality of *Callosobruchus maculatus* reaching 100%. In addition, flower extracts showed their repellency potential against *Sitophilus zeamais* when applied to corn grains and weight loss (3.28 ± 0.45%) [21]. Ambrosio et al*.* [22] reported that dichloromethane leaves rinse extract at a concentration of 5% of fresh leaves, has antifeedant potential on *Chlosyne lacinia*. Nthoingambi and Singh (2013) showed the antifungal and antibacterial efficiency of petroleum ether, chloroform and methanol extract of *T. diversifolia*, using a poisoned food technique and a disc diffusion method, respectively. Petroleum ether extract displayed the most potent antifungal activity, by inhibiting all the fungi by confrontation tested or by diffusion with well disc method against some phytopathogens as *Alternaria solani*, *Aspergillus niger*, *Drechslera oryzae*, *Fusarium oxysporum.*

3.1.3 Antioxidant

T. diversifolia extracts displayed cytotoxic effects with half maximal inhibition (IC_{50}) values of 3.02 to 12.82 µg/mL at variable pathogen cells. It also showed radical scavenging activities on DPPH (1,1-diphenyl-2-picrylhydrazine) and ABTS (2,2-Azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid) radicals, with IC_{50} values of 108.8 and 41.7 µg/mL, respectively [23]. However, the antioxidant capability of *T. diversifolia* extracts have been shown by its sequestering capacity on DPPH by reacting with free stable radical to form DPPH [24]. The aqueous and ethanolic concentrations of extracts causing IC_{50} were respectively evaluated around 2.27 and 0.63 mg/mL. The aqueous extract of *T. diversifolia* also elicited a good antioxidant property, with an N-acetyl cysteine equivalent antioxidant capacity of 32.62 and 20.99 mg of N-acetyl cysteine/g for ABTS and DPPH radical assays, respectively [25]. In addition, *T. diversifolia* is a good source of phenolic antioxidants and can be used to manage oxidative stress associated to illnesses [26] Ethyl acetate (IC_{50}: 30.81 ± 0.48 µg/mL) extracts of *T. diversifolia* exhibit a good inhibition against acetylcholinesterase and butyrylcholinesterase. *T. diversifolia* extract shows higher inhibitions than galantamine (IC_{50}: 42.20 ± 0.44 µg/mL) against butyrylcholinesterase. Equally, good inhibitions are showed on α-amylase and α-glucosidase. Table 1 highlights the importance of some antioxidant molecule activities in the growth and the protection of the plant.

Table 1. Antioxidants: importance in the plant

Antioxidants	Role in plant
Ascorbic Acid	Allows the development of tissues and the plant, it is related to the respiratory intensity of the plant.
Pyridoxin	Involved in the synthesis and degradation of amino acids and proteins.
β-Caroten	Absorbs the full energy of the chlorophyll in order to avoid the formation of reactive oxygen species which would destroy the leaves.
Tocopherol	Promotes fertility.
Phenolic compounds (phenolic acids, flavone, chlorogenic acid, quinones…)	Antimicrobial power, they participate in the defense of the plant, in the reinforcement of the walls of the plant cells and in their capacity to modulate and induce the defense reactions of the host.
Lycopen	Responsible for the color of the plant, flowers, fruits, leaves ...

3.2 Phytochemical Studies on *T. diversifolia* Extracts

3.2.1 Global composition of *T. diversifolia*

Analysis of the leaves, stems, flowers and roots of *T. diversifolia* have shown the presence of carbohydrates, crude fibers, moistures, total ashes, crude proteins and crude fats in the parts of the plant. Crude fiber contents are high in stems followed by carbohydrates in leaves and fat in stems [27]. Leaves and roots contain high nutritive substances values that can be explored for several purposes, such as agricultural and pharmacological ones. All *T. diversifolia* organs are employed in the management of soil fertility, plant growth promotion and biocontrol of diseases, thanks to the biological active components. Moreover, secondary metabolites contained in the extracts as totals proteins and phenolic compounds, are involved in physiological role of plants. Table 2 shows the presence of secondary metabolites depending on the different plant parts and the solvents used, thanks to the analysis of the crude extracts of *T. diversifolia*. Leaves of the studied plant contain the higher number of metabolites, compared to the other plant parts. Yet, saponins in acetone extracts, steroids in aqueous extracts and terpenoids in methanol and acetone extracts are not present in the leaves. Moreover, phytochemical and mineral analysis have shown, in the different parts of the plant, the presence of secondary metabolite contents such as phenols, terpenoids, flavonoids, alkaloids, tannins, saponins (Olo'o and Mengue, 2020). The green leaf biomass of *T. diversifolia* is higher in nutrients compared to a dry matter basis of some agroforestry species, whose leaves are most often used for biofertilization in crops (Table 3). The following mineral element contents of *T. diversifolia* are clearly superior, compared to those of the main agroforestry species used to improve productivity: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (Table 3). This Ability of *T. diversifolia* depends on the biomass yield, their quality and their rate of decomposition [17].

3.2.2 Phytochemicals constituents of *T. diversifolia*

T. diversifolia extract efficiency is important but results could be improved. Indeed, a better knowledge of chemistry and biological activities, such as fertilization and stimulation, could

Table 2. Phytochemical constituents in leaves, stems and roots of *T. diversifolia* (Olo'o and Mengue, 2020)

		Extraction		
Phytochemicals constituents	Plant parts	Methanolic	Acetone	Aqueous
Saponins	Leaves	+	-	+
	Stems	-	-	+
	Roots	-	-	+
Flavonoids	Leaves	+	+	+
	Stems	+	+	+
	Roots	+	+	+
Alkaloids	Leaves	+	+	+
	Stems	+	+	+
	Roots	+	-	+
Steroids	Leaves	+	+	-
	Stems	-	-	+
	Roots	+	-	-
Terpenoids	Leaves	-	-	+
	Stems	-	+	-
	Roots	-	-	-
Tannins	Leaves	+	+	+
	Stems	+	+	+
	Roots	+	+	+
Glycosides	Leaves	+	+	+
	Stems	+	-	+
	Roots	+	-	-

Keys: (+) Presence of metabolites; (-) Absence of metabolites

Table 3. Major's minerals contents of leaves of *T. diversifolia* compared to agroforestry species [17]

Species	Concentration (%)				
	N	P	K	Ca	Mg
T. diversifolia	3.53	0.42	4.70	3.52	0.45
D. intortum	1.79	0.30	0.58	1.70	0.28
P. phaseoloides	2.17	0.37	0.59	2.75	0.32
C. calothyrsus	3.40	0.15	1.10	Nd	nd
L. camara	2.80	0.25	2.10	Nd	nd
T. vogelli	3.00	0.19	1.00	Nd	nd

nd: not determined.

Table 4. Phytochemical constituent quantities of *T. diversifolia* according to plant organs [17]

Composition (mg/100g)	Organs			
	Leaves	**Stems**	**Roots**	**Mean**
Alkaloids	1535.00	361.67	863.33	853.33
Tannins	540.00	125.00	481.67	382.22
Flavonoids	851.67	33.33	131.67	338.89
Saponins	761.70	38.33	183.33	327.78
Terpenoids	126.67	18.33	50.00	65.00
Totals phenols	64.58	9.77	71.03	48.46

enhance the identification of bioactive components. The bioactivity is due to a single or a mixture of compounds, which contributes to additive or synergistic effects [28]. The great challenge is to characterize most of the complex compounds in the fermented liquid extract, to understand this mixture effect. This would lead to a phytochemical fingerprinting of the extract. A phyto-complex consisting of a combination of both substances: an active principle and a mixture of the three parts of the plant (leaves, stems, roots). This phyto-complex can contribute to the overall biological effects [29,30]. Many phytochemical constituents have been identified in aqueous and ethanol extracts of leaves, stems and roots of *T. diversifolia* plant [17]. Table 4 shows that leaves have a good accumulation of secondary metabolites compared to stems and roots.

3.2.2.1 Terpenoids

Terpenoids are the largest and most diverse group of secondary metabolites derived from *T. diversifolia*. Several bioassays refer to the therapeutic potentials of terpenoids against parasitic diseases. The versatility of the parent terpenoid backbones allows structural diversity among the group. This property leads to multiple cellular targets and consequently varied mechanisms of antiparasitic actions. Terpenoids are the most common metabolites in *T. diversifolia*, of which sesquiterpenes is the most available. These sesquiterpenes are the largest group of secondary metabolites, which have a C_{15} skeleton formed by three isoprene units [31]. The oxidation of one isoprene unit into a methanol group conducts to a lactone. Sesquiterpene lactones are well known as the most varied class with over 35,000 known structures. Many of these sesquiterpenes have high biological activities [32]. They synthesize in the plant terpenoids as *Asteraceae*. They also play an important role in plant defense, as antibacterials, antifungals and insecticides agents.

The phytochemical investigation leading to the identification and the classification revealed that the major sesquiterpenoid groups such as furanoheliangolides, eudesmanolides, guaianolides and germacranolides, are the most studied [33-35]. Extracts obtained by hydrodistillation presented components of essential oils. Forty-five compounds were identified and characterized in the non-volatile fraction compared to more than fifty complex compounds in volatile fraction. The tagitinine is one of the mostly known sesquiterpene lactone isolated of interest. Many more sesquiterpenes lactones are related to some biological activities as antifeedant activities on several arthropod pests [36,37].

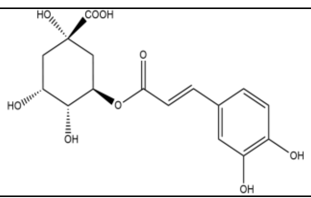
3.2.2.2 Phenolic compounds

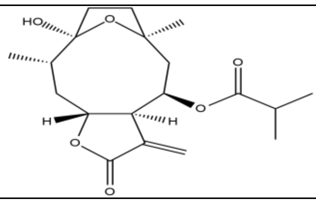
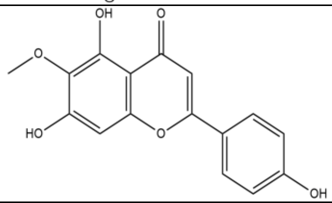
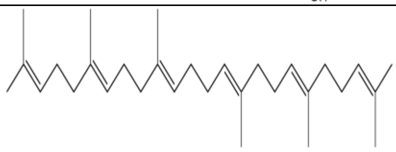
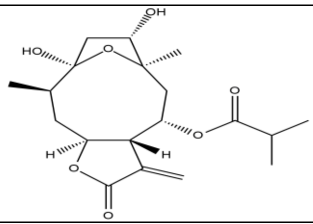
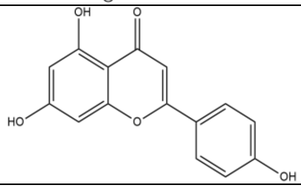
Phenolic compounds, known to be contained in *T. diversifolia* plant, belong to its major bioactive constituents (Chagas-Paula et al*.* 2011) [22,31]. The reports have shown the effectiveness of some phytochemical compound contents in *T. diversifolia.* These compounds are phenolic acids and various classes of flavonoids, present in the dry powder form and related to rice meal moth and *Corcyra cephalonica* (Ojo et al*.,* 2020) [38]. Polyphenols react like chemical entities that prevent Reactive Oxygen Species (ROS). ROS induce oxidative damage by inhibiting the enzymes involved in carbohydrate hydrolysis [39]. Studies have reported that *T. diversifolia* extracts were a good source of natural antioxidants. Studies also demonstrated their usefulness as medicinal supplements and alternative drugs, in the green synthetic processes of metallic nanomaterials [40].

3.2.3 Bioactive compounds of *T. diversifolia* extracts

Generally, *T. diversifolia* fermented extracts based on bioactive compounds are exploited in a sustainably manner. They can be an alternative source of biological and eco-responsible agriculture, as well as an affordable and accessible natural biostimulant for smallholders and compagnies. These compounds would require the characterization in the fermented extract to provide new information about their potential. Complete characterization of an extract is possible for commonly used plants, because chemical constituents are not all well-known in *T. diversifolia* extract (Table 5). *T. diversifolia* fermented extract which could be influence by fermentation duration protocol in bioactive compounds extraction come from microbial active fraction as metabolic product and organic part concern a non-degraded vegetal material. The extract can be used by soil or foliar applications, which will be the guideline of this work [41]. The variations of agro-environmental conditions can influence accumulation of phytochemicals constituents in *T. diversifolia* extract, or can make a lot of complex structure in characterization of extract. These variations are proved by Liquid Chromatography-Mass Spectrometry (LC–MS); Nuclear Magnetic Resonance (NMR) and Flamme Atomic adsortion Spectroscopy (FLAA) techniques. Even the most sensitive and advanced method of metabolite profiling, an exploration of results would generate a long list of complex not identified compounds or new identified compounds inside extract probably in *T. diversifolia* database. Several analytical techniques have been used on crude extracts in order to characterize plant species as sources of biological active compounds. So, large amounts of fresh vegetal materials are initially used to identify a single compound. The identification depends on the chosen extracting solvent [42]. The appreciated method to analyze the extract involves an efficient High Performance Liquid Chromatography (HPLC) with a pressure control and an isocratic system, to reduce the running time and optimize separations. Reverse phase chromatography using C_{18} as stationary phase, is able to separate compounds found in the extracts. The resolution of mass spectrometry achieved with liquid chromatography allows the detection and quantification of a large number of molecules in a single measurement. Various ionization methods can be utilized with MS, but Electron Spray Ionization (ESI) is the most adapted here. ESI is preferable because this ionization preserve molecular ions and allow identification of unknown compounds [43]. In addition, the NMR 2D experiments can provide a useful phytochemical fingerprinting" on a range of compounds present in crude extracts [44]. Nowadays, studies have not been yet reported or executed for the *T. diversifolia* fermented extract and its bioactive compounds. Its broken-down metabolites could increase the microbial properties as well their biostimulant effects.

Table 5. Some biologicals properties of *T. diversifolia* constituents and their applications

Chemical compounds	Structures	Biological properties	Application fields (References)
Chlorogenic Acid $C_{16}H_{18}O_9$		A nutritional antioxidant in plant-based foods. Inhibits irreversible hydrolysis of G-6-Pase (reduction of glycogenolysis).	Antioxidant activity, antibacterial, antiviral, anti-microbial (Ojo et al., 2018)

Chemical compounds	Structures	Biological properties	Application fields (References)
Tirotundin $C_{19}H_{28}O_6$		Potential neurotoxins to nematodes.	Nematicid Activity (Lan et al., 2022)
Hispidulin $C_{16}H_{12}O_6$		Inhibits glutamate release in benzodiazepine receptor ligand nerve endings to the allosteric-positive.	Antifungal activity (Baruah et al., 1994)
Squalen $C_{30}H_{50}$		Intermediate of sterol and hopanoid biosynthesis in various types of cells from bacteria.	Antioxydant Adjuvant Vaccinal (Ragasa et al., 2007)
Tagitinin A $C_{20}H_{31}O_7$		Reduces lipopolysaccharides induced by interleukins 6, 8 and tumor necrosis α factors.	Potential botanical insecticide Antifeedant activity [45]
Apigenin $C_{15}H_{10}O_5$		Insect feeding Deterrent repellent agent CYP2C9 inhibitor (responsible for drug metabolism).	Insecticide activity (Ojo et al., 2018)

3.3 Stimulatory Effect of *T. diversifolia* on Plantain Banana Plants

Recent studies carried out in Cameroon have demonstrated that *T. diversifolia* extracts are used as biofertilizer and biocontrol agents. These agents involve different application forms of the plant parts extracts. Different treatment applications based on extracts have been developed to promote the plant growth and to decrease the risk of Black Sigatoka Disease: mulching or mixing with powder or dry form, watering with fermented liquid form on the PIF seedlings. Dried leaves and stems of *T. diversifolia* have been used as substrate amendment on plantain banana and have permitted to obtain vigorous vivoplants. Both leaves and stems have shown better results with 56% treated plants compared to control ones [46]. Indeed, the bioefficiency of *T. diversifolia* dried mulch is stimulant in the plant growth of plantain banana PIF seedlings in the nursery and prevent the *M. fijiensis* apparition. It also enhances the accumulation of biomarkers such as proteins, polyphenols and defense-related enzymes such as peroxidase, polyphenol oxidase and glucanase [12]. *T. diversifolia* fermented liquid extracts were found to significantly influence the vegetative and germination stages of PIF plantain bananas seedlings at 21 first days after lunching method compared for the control ones. So, the germination has reached 100% for treated plants against 66% for controls ones. Concerning growth parameters, their average increase by 27% in diameter, 27% in the length of the pseudo stems and 30% in the photosynthetic leaf area. Treated seedlings showed maximum protection against Black Sigatoka Disease beyond 87% compared to seedling controls [6]. However, in the field stage with the mature plantain banana tree, fermented liquid base on *T. diversifolia* extract showed their bioefficiency with biopesticidal potential to reduce the severity of Black Sigatoka Disease in the course of time, and confirmed by the very low concentration [47].

T. diversifolia mulch stimulates the plant growth, directly on PIF plantain banana seedlings physiology, and indirectly on substrate in nursery [12]. Effectively, this organic amendment has modified the physical properties of the soil such as the stability of aggregates and the porosity that can improve the roots growth, rhizosphere and stimulate plant growth. In the same way, vegetative growth parameter on plantain has been increased by the treatment of liquid extract of *T. diversifolia*. Beneficial effects of fermented liquid extracts begin with the amelioration of the soil properties [48,49]. This involves physicochemical and biological characteristic modifications as microbiota around plant roots to better assimilate uptakes. Root assimilations, length development and leave area development are observed on treated plantain banana seedlings compared to controls. It shows the potential stimulatory role of liquid extract to increase the availability of assimilated nutrients during the growth. This efficiency on plantain banana is confirmed by Tatsegouock et al [6], with 87% of the plant protection and the good accumulation of biomarkers concerning growth promotion in leaves with 900 to 1200 mm^2 of foliar area. However, *T. diversifolia* is an excellent biostimulation tool on induction of plant defense. It improved defense responses against phytopathogens such as *Mycospharella fijiensis* in terms of severity of the black sigatoka disease. The level of the protection rate for the plantain banana achieved 83% when the plant has been amended with mulch of dry form *T. diversifolia*, according to Meshuneke et al. [12]. Comparatively, this level reached 87% with the liquid extract obtained, according to Tatsegouock et al. [6]. So, the reinforcement of the plant cell wall, which is the first mechanism started during plant attack, can be quick observed when the cultivated plant is watering with the extract [11]. Indeed, specific information about the action mechanism of liquid extracts, their implication to this protection and the compounds responsible for their pesticidal effects are not yet known. Treatment applications based on fermented liquid extracts do not yet fully demonstrate the phytochemicals activities of the constituents and the effectiveness in Black Sigatoka Disease biocontrol [50-52]. The phytochemical knowledge of this aqueous and fermented liquid extract against *M. fijiensis* or other several phytopathogenes is therefore still mulch wanting. However, the same metabolites or biomarkers are sometimes involved in the growth promotion and defense induction on plantain banana seedlings [53-55].

4. CONCLUSION

T. diversifolia works as a biostimulant product for increasing crop productivity. The current review highlights the stimulatory effect of this product as a biofertilizer and a pesticide. It has antioxidant activities and some plant protections. The health-related aspects of some extracts are linked to their phytochemical constituents. Recently, most published biostimulant applications have shown that extracts from fresh material, are more widely used than pure isolated compounds for some biological activities. Molecules such as phenolic compounds, terpenoids, alkaloids and essentials oils are mobilized secondary metabolites involved in the plant defense mechanisms against pathogens and in growth promotion. These compounds are also involved in the defense responses of the banana tree, although their mechanisms of action joined to their properties are not yet known and well understand. The action mechanism of these extracts or phytochemical constituents are not fully demonstrated. Thus, there is still a lack of their stimulatory activity knowledge too. The challenges for identifying and characterizing fermented liquid extracts that comprise a multitude of compounds, should be solved by high-performance liquid chromatography coupled to mass spectrometry analysis. The major bioactive compound with the greater stimulatory potential of the *T. diversifolia* fermented liquid extract, and the mechanism of action should be comprehended further. By the way, these points need to be understood for the global acceptance of this product in eco-agriculture and later in human health, before labeling.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologsies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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