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Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-12, December 2018, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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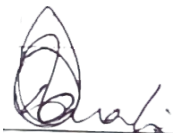

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Physicochemical Characterization of African Aubergine *Solanum Aethiopicum Anguivi* (Solanaceae) from Northern Cote d'Ivoire

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Abstract— *Solanum aethiopicum anguivi (Solanaceae) is a common vegetable widely used for food by population in Korhogo, northern Côte d'Ivoire. But this aubergine is not yet soundly utilized. The current study focuses the physico-chemical traits of this aubergine to fit more valorization. From the investigation, the aubergine showed higher moisture (90.73% to 92.71%). Oppositely, lower contents are recorded for crude proteins (1.44% to 1.64%), fats (0.12% to 0.16%), total carbohydrates (5.38% to 6.71%), and so for caloric energy value (28. 36 Kcal/100 g to 34.84 Kcal/100g). Besides, the aubergine is provided with significant fiber content (2.43% to 3.31%) and displayed antioxidants components such as vitamin C (6.25 mg/100 g to 6.74 mg/100g) and polyphenols (55.94 mg/100 g to 66.34 mg/100g). It also presents 0.36% to 0.76% of ash mainly constituted of potassium (4.52% to 5.42% DM), phosphorus (0.82% to 0.97% DM) and other oligoelements as iron (1.42 to 4.81 ppm), manganese (2. 06 to 2.33 ppm), zinc (0.22 to 0.32 ppm), and copper (0.01 to 0.02 ppm). Still, this vegetable records phytate (20.91 to 22.44 mg/100 g) and oxalate (28.69 to 38.83 mg/100 g) as main antinutrients components. Processed before consumption, Solanum aethiopicum anguivi constitutes a significant source of food fibre, natural antioxidant, and mineral elements for local population*.

Keywords— Aubergine, Physico-chemical properties, Solanum aethiopicum anguivi.

I. INTRODUCTION

African aubergine is one of the most commonly grown and consumed fruit vegetables in tropical Africa. It's the third largely consumed vegetable after tomato, onion and okra. The annual production of the fruit is 60 000t in Côte d'Ivoire [1]. The aubergine Solanum aethiopicum anguivi is a vegetable plant of the Solanaceae family grown for its fruit, which are borne 3 to 5 months over planting [2], and consumed as vegetable. The interest of vegetable plants in food for populations is very widely recognized in the world [3, 4]. In Côte d'Ivoire, the aubergine fruits and leaves are eaten.

In Korhogo, northern Côte d'Ivoire, the species *Solanum aethiopicum anguivi* is grown in several areas like villages and usually sold on the markets. This aubergine is used by populations for feeding, for the preparation of sauces and consumed with rice and pounded meals such as local foufou or foutou.

Nevertheless, the aubergine Solanum aethiopicum anguivi is under-exploited and the incomes recovered by stakeholders, namely farmers and sellers from markets, are meager. Yet, significant amounts of aubergine still rot during the distribution channel before being purchased by the consumers. Conservation problems prevail at the level of the distribution chain, a better control of the physico-chemical and nutritive parameters could contribute to ensure a better profitability of the aubergine. In addition, the populations are not soundly aware of the nutrients provided by the aubergine. This work is a valorization of the species Solanum aethiopicum anguivi by the determination of its physicochemical properties.

II. MATERIAL AND METHOD

2.1 Plant material

The study was performed on fruits of aubergine *Solanum aethiopicum anguivi* collected from Korhogo in northern Côte d'Ivoire.

2.2 Methods

2.2.1 Sampling

The aubergine samples were purchased from three (3) main markets of Korhogo City: Sinistré market, Soba market, and Koko market. Per market, the aubergine samples were purchased from three various sellers, four (4) kg each. Thus, 12 kg of aubergine were gathered per market, leading to 36 kg for overall samples purchased. The samples were then conveyed into laboratory for further analyses.

2.2.2 Physical Characterization of *Solanum aethiopicum anguivi*

Five (5) physical parameters were assessed on the aubergine fruits, namely length, circumference, weight, moisture, and ash.

The length and the circumference of the full fruit were estimated using a meter tape. The fruit's weight was measured using a 2 digits scale (Sartorius,).

The method of determining moisture is that proposed by AOAC [5]. The moisture was assessed by drying 5 g of aubergine into an oven at 105 °C till constant weight resulted after 24 h.

The ash content was measured by incinerating five (5) g of oven-dried aubergine into a muffle furnace at 550 °C for 12 h [5].

2.2.3 Chemical trend of the aubergine fruits

A. Acidity

The acidity traits (pH and titratable acidity) were measured using **AOAC** method [5]. Ten (10) grams of crushed sample are slurried in 100 mL of distilled water. The solution obtained is filtered on filter paper (Whatman). The pH measurement is carried out directly by immersing the previously calibrated pH meter (HANNA) electrode in the filtrate obtained. Then, 10 mL of the filtrate are taken and this test sample is titrated with a solution of NaOH (0.1 N) in the presence of phenolphthalein until turning pink. The titratable acidity is given in mEq/100g of dried sample.

B. Total soluble carbohydrates and reducing carbohydrates contents

Ethanosoluble carbobydrates were extracted from 1 g of ground dried aubergine with 20 mL of 80% (v/v) ethanol, 2 mL of 10% (m/v) zinc acetate and 2 mL of 10% (m/v) oxalic acid, according to the method of Agbo *et al*. [6]. The extract was centrifuged at speed of 3,000 rpm for 10 min. The ethanol residue was evaporated from the extract upon a hot sand bath.

Then, the extracted total soluble carbohydrates were measured out using the method of Dubois *et al*. [7]. The operation consisted in adding 0.9 mL of distilled water, 1 mL of 5 % (m/v) phenol, and 5 mL of 96% sulfuric acid into 100 µL of extract, then measuring the absorbance at 490 nm with a spectrophotometer (PG instruments). For the reducing sugars, 1 mL of extract was processed with 0.5 mL of distilled water and 0.5 mL of 3, 5- dinitrosalycilic acid [8] prior to the recording of the absorbance from the final solution at 540 nm with a spectrophotometer (PG instruments).

Calibrations were performed with standard solutions of glucose and sucrose for recovering the final total carbohydrates and reducing carbohydrates contents in the studied samples.

C. Lipids content

Lipids were quantified from 10 g of ground dried aubergine sample by solvent extraction using 300 mL of n-hexane reagent and a Soxhlet device for 7 h [9]. The hexan-oil mixture resulted from the extraction was recovered and separated with a rotavapor apparatus (Heidolph). The difference between the sample weight before and after the experiment allowed the estimation of the lipids content.

D. Proteins content

Crude proteins content was determined as the total nitrogen using the Kjeldhal method [5]. Thus, 1 g of aubergine mash was mineralized at 400 °C for 2 h, with adding of concentrated sulfuric acid (H_2SO_4) and potassium sulfate (K_2SO_4) catalyst. The mineralizate was diluted and distilled for 10 min. Thereafter, the distillate collected into a flask containing boric acid and

methylen bromocresol reagents ion, was titrated for the total nitrogen using ammonium sulfate ($(NH_4)_2SO_4$). The crude protein content of the aubergine was deduced from the nitrogen level using 6.25 as conversion coefficient.

E. Fibers content

The determination of the crude fibers content consisted in treatment of 2 g of ground aubergine sample with 50 mL of 0.25 N sulfuric acid and 50 mL of 0.31 N sodium hydroxide and filtration of the resulting solution upon Whatman paper. The residue was dried for 8 h at 105 °C then incinerated at 550 °C for 3 h into ovens [10]. The final residue was weighed as crude fibers and expressed in percentage.

F. Total carbohydrates content and energy value

Total carbohydrates and energy values were determined using calculation formulas [11] accounting the moisture, fat, protein, ash contents and the energy coefficients for macromolecules.

$$TCC\ (\%) = 100-[P(\%) + M(\%) + F(\%)+A(\%)] \qquad CEV\ (kcal/100g) = [(4\times P) + (9\times F) + (4\times C)]$$

With: TCC, total carbohydrates content; CEV, caloric energy value; P, protein content; M, moisture content; F, fat content; A, ash content; C, total carbohydrates content

G. Vitamin C content

The vitamin C was evaluated from the aubergines using 2,6- dichlorophenol-indophenol (DCPIP) reagent [12]. Ten (10) grams of ground dried aubergine sample were dissolved into 40 mL of metaphosphoric acid-acetic acid solution (2%, w/v). The resulted mixture was centrifuged at 3,000 rpm for 20 min. Thus, the supernatant was recovered, added with boiled distilled water for 50 mL, and titrated with 2, 6- DCPIP solution (0.5 g/L) previously calibrated with a pure vitamin C solution.

H. Oxalates content

The oxalate content was determined with the standard AOAC method [5]. Two (2) grams of ground dried aubergine sample were homogenized into 200 mL of distilled water and added with 20 mL of 6N hydrochloric acid (HCl). The mixture was heated in boiling water bath for 1 h, cooled, and filtered. Fifty (50) mL of the filtrate were then homogenized into 20 mL of 6 N HCl, and filtered again. The 2nd filtrate was treated with methyl red (0.1%, w/v), concentrated ammonia, heated, and filtered. The 3rd filtrate was boiled, treated with calcium chloride (5%, w/v) for the formation of calcium oxalate crystals, and then filtered once more. The residues deriving from the filtration steps were successively washed with distilled boiling water, dried into an oven; dissolved into 10 mL of diluted sulfuric acid, and titrated with 0.05N potassium permanganate solution.

I. Phytates content

The phytates were measured according to the method processed by Mohammed *et al*. [13]. A slight ground aubergine sample (0.5 g) was treated with 25 mL of TCA solution at 3% (w/v) and centrifuged at 3,500 rpm for 15 min. Five (5) mL of the supernatant was removed, treated with 3 mL of ferric chloride 1% (w/v) reagent, heated in a boiling water bath, cooled and also centrifuged at 3,500 rpm for 10 min. The 2nd supernatant was treated with 5 mL of 0.5N hydrochloric acid, 5 mL of 1.5N sodium hydroxide, heated in a boiling water bath and centrifuged once more at 3,500 rpm for 10 min. Thus, 1 mL of the final supernatant was added with 4.5 mL of distilled water and 4.5 mL of orthophenantroline reagent and then measured for the absorbance at 470 nm with a spectrophotometer against standard Mohr salt solution treated likewise and taken as phytates ferric control.

J. Polyphenols contents

The phenol compounds were extracted from aubergine with methanol reagent. One gram of dried aubergine sample was homogenized in 10 mL of methanol solution 70% (v/v). The resulting mixture was centrifuged at 1,000 rpm for 10 min. The pellet was recovered and treated likewise. The deriving supernatants were thus gathered into a marked flask and added with distilled water at 50 ml.

The total polyphenols content was measured using Folin-ciocalteu reagent, sodium carbonate solution (20% w/v) and distilled water [14]. Essays were measured for their absorbance at 745 nm with a spectrophotometer against standard gallic acid solutions taken as polyphenols control.

The tannins content was deducted from the total polyphenols using vanillin reagent [15]. Essays were measured for their absorbance at 500 nm with a spectrophotometer against standard tannic acid solutions taken as tannins control.

Flavonoids content was also determined from the total polyphenols using aluminum chloride (10% w/v), potassium acetate (1 M) and distilled water [16]. Essays were measured for their absorbance at 415 nm with a spectrophotometer against standard quercetin solutions taken as flavonoids control.

K. Determination of mineral elements

The determination of the mineral elements was performed according to the IITA method [17]. Finely ground aubergine sample (0.4 g) previously oven dried at 60 ° C was incinerated into a muffle furnace at 550 °C for 3 h. The resulting gray-white ash was cooled, added with 2 mL of half-diluted HCl, placed on a sand bath at 120 °C until full evaporation, and then ovened at 105 °C for a 1 h. The final dry extract was recovered with 2 mL of half-diluted HCl, filtered, and the resulting filtrate added with distilled water, and lanthanum chloride. The mineral elements in the solution were then measured using Atomic Absorption Spectrometry (AAS 20 type VARIAN).

2.2.4 Statistical analysis

All chemical analyses and essays were performed in triplicate. The data were statistically analyzed using Statistical Program for Social Sciences software (SPSS 22.0, USA). The statistical treatment consisted in a one-way analysis of variance (ANOVA-One way) according to market of perception of the aubergine taken as source of variation at 95% significance. Then, the means were compared using Student Newman Keuls post-hoc test.

III. RESULTS

3.1 Physical characteristics of *Solanum aethiopicum anguivi*

The aubergine S. aethiopicum anguivi displays an oval shape. Both aubergines show invarious medium circumference (7.91 cm), length (4.38 cm) and weigh (9.05g) general average contents. Oppositely, table I shows higher moisture (92.71%) for aubergine from Koko market compared to the respective 90.73% and 91.86% moisture of aubergine from Sinistré and Soba market and higher ashes (0.76%) for aubergine from Sinistré market compared to the respective 0.39% ash and 0.36% ash for aubergine from Soba and Koko market.

TABLE 1
SOME PHYSICAL PARAMETERS OF *SOLANUM AETHIOPICUM ANGUIVI*

Parameters	Sinistré market	Soba market	Koko market	$F_{\text{-value}}$	$P_{\text{-value}}$	GA
Shape (cm)	Oval	Oval	Oval	-	-	-
Length (cm)	4.30 ± 0.40^{a}	4.46 ± 0.40^{a}	4.38 ± 0.24^{a}	0.163	0.853	4.38±0.29
Circumference (cm)	7.64 ± 0.49^{a}	8.25 ± 1.57^{a}	7.83 ± 0.39^{a}	0.300	0.751	7.91±0.84
Mass (g)	8.35 ± 0.08^{a}	9.97 ± 2.14^{a}	8.84 ± 0.05^{a}	1.349	0.328	9.05±1.22
Moisture (%)	90.73 ± 0.45^{c}	91.86 ± 0.08^{b}	92.71 ± 0.05^{a}	41.15	**<0.001**	
Ashes (%)	0.76 ± 0.02^{a}	0.39 ± 0.01^{b}	0.36 ± 0.01^{c}	577.8	**<0.001**	

Per raw, values with various lower scripts are different at 5% significance. $P_{\text{-value}}$, statistical value of the probability test; F-value, statistical value of the Ficher test; GA, general average

3.2 Chemical characteristics of *Solanum aethiopicum anguivi*

Except for the pH, where aubergines from Koko market provide more value (8.60), the main chemical traits show that aubergine from Sinistré market provide more acidity value (0.98 mEq/100 g), more protein (1.64%), lipid (0.16% w/w), total

carbohydrates (6.71% w/w), total fiber (3.31% w/w), total sugar (2.97% w/w), reducing sugar (0.13% w/w) contents and more energy value (34.84 Kcal/100g) (Table II).

3.3 Main micronutrients, polyphenols compounds and antinutrients of *Solanum aethiopicum anguivi*

The aubergines studied from the three markets show invarious contents in vitamin C, phosphorus, potassium, calcium, magnesium, sodium, magnesium, zinc, copper, phytates, with respective general average of 6.55 mg, 0.88% DM, 4.93% DM, 0.48% DM, 0.52% DM, 6.49 ppm, 2.20 ppm, 0.27 ppm, 0.02 ppm, and 21.58 mg. Oppositely, the table III shows higher contents in total polyphenols (66.34 mg), tannins (51.75mg), flavonoids (0.55mg), iron (4.81ppm), oxalates (38.83 mg) for aubergine collected from Sinistré market.

TABLE 2
MAIN CHEMICAL COMPOSITION OF *SOLANUM AETHIOPICUM ANGUIVI*

Parameters	Sinistré Market	Soba Market	Koko Market	$F_{\text{- value}}$	$P_{\text{-value}}$
pH	6.17 ± 0.01^{c}	7.37 ± 0.02^{b}	8.60 ± 0.01^{a}	36336.4	<0.001
Acidity	0.98 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	$2.921.10^{33}$	<0.001
Proteins (%w/w)	1.64 ± 0.04^{a}	1.56 ± 0.01^{b}	1.44 ± 0.04^{c}	33.23	0.001
Lipids (%w/w)	0.16 ± 0.01^{a}	0.14 ± 0.01^{b}	0.12 ± 0.00^{c}	18	0.003
Total carbohydrates (% w/w)	6.71 ± 0.40^{a}	6.07 ± 0.05^{b}	5.38 ± 0.11^{c}	22.6	0.002
Total fiber (%w/w)	3.31 ± 0.33^{a}	3.32 ± 0.1^{a}	2.43 ± 0.09^{b}	19.34	0.002
Total sugars (%w/w)	2.97 ± 0.16^{a}	2.66 ± 0.06^{b}	2.23 ± 0.06^{c}	37.95	<0.001
Reducing sugars (%w/w)	0.13 ± 0.01^{a}	0.10 ± 0.01^{b}	0.06 ± 0.01^{c}	52	<0.001
Energy value (Kcal/100g)	34.84 ± 1.47^{a}	31.78 ± 0.27^{b}	28.36±0.36	40.329	<0.001

Per raw, values with various lower scripts are different at 5% significance. P-value, statistical value of the probability test; F-value, statistical value of the Ficher test

IV. DISCUSSION

The moisture content of the aubergine Solanum aethiopicum anguivi is high. This percentage is close to the moisture value of tomato variety ''Tounvi'' (94.97%) reported by **Dossou *et al.* [18]**, and higher than that of mango (83-85%) found by Touré and Kibangou-Nkembo [19]. The higher moisture content of aubergine forecasts a rapid change in post-harvest state such as rotting [20].

For the physicochemical composition of the aubergine studied, there was a slight difference between the levels according to the markets. This is due to the slight difference in moisture content; the higher the moisture, the lower the contents in proteins, lipids, carbohydrates, ashes, etc. The protein content (1.44 to 1.66%) is greater compared to the 0.18% found from the ginger starch [21]. The lipid content (0.12 to 0.14%) is close to that recorded in cooked carrot (0.1%) and cucumber (0.11%) by Ciqual [22]. The total carbohydrate content (5.38 to 6.71%) is lower compared to the 18.9% carbohydrates values of potato [23].

Ultimately, the aubergine contains macromolecules (carbohydrates, lipids, proteins) essential for life, albeit in small quantities. It could be recommended for persons against gain of weight. The percentage of fiber (2.42 to 3.31%) is close to that of the fresh dough of young shoots tubers of *Borassus aethiopum* (3.92%) [24]. Aubergine may be a significant source of dietary fiber that is removed more slowly from the stomach and improves bowel movement. These dietary fibers are absolutely essential to the balance of the digestive tract and that of the body. They represent a factor of good health. Studies have shown an inverse correlation between dietary fiber consumption and colon cancer.

TABLE 3
POLYPHENOLS, MICRONUTRIENTS AND ANTINUTRIENTS OF *SOLANUM AETHIOPICUM ANGUIVI*

Parameters		Sinitré Market	Soba Market	Koko Market	F_{-value}	P_{-value}	General average
Polyphenols compounds (mg/100g)	**Total phenols**	66.34±0.55[a]	64.47±1.23[b]	55.94±0.15[c]	150.596	**<0.001**	
	Tannins	51.75±1.78[a]	34±.71±1.00[b]	30.91±0.21[c]	264.414	**<0.001**	
	Flavonoids	0.55±0.01[a]	0.39±0.01[b]	0.34±0.02[c]	206.467	**<0.001**	
Vitamin and mineral elements	**Vit C (mg/100g)**	6.74±0.30[a]	6.66±0.51[a]	6.25±0.34[a]	1.340	0.330	6.55±0.36
	P (%DM)	0.97±0.16[a]	0.85±0.42[a]	0.82±0.03[a]	0.257	0.781	0.88±0.22
	K (%DM)	5.42±0.24[a]	4.85±0.05[a]	4.52±1.57[a]	0.743	0.515	4.93±0.84
	Ca (%DM)	0.71±0.26[a]	0.42±0.05[a]	0.32±0.04[a]	5.085	0.051	0.48±0.20
	Mg (%DM)	0.57±0.07[a]	0.52±0.08[a]	0.46±0.03[a]	2.411	0.172	0.52±0.06
	Na (PPM)	7.41±0.33[a]	6.35±2.14[a]	5.70±0.84[a]	1.240	0.354	6.49±1.30
	Fe (PPM)	4.81±0.39[a]	3.54±0.54[b]	1.42±0.05[c]	58.759	**<0.001**	
	Mn (PPM)	2.33±0.23[a]	2.21±0.19[a]	2.06±0.04[a]	1.754	0.251	2.20±0.20
	Zn (PPM)	0.32±0.16[a]	0.28±0.17[a]	0.22±0.02[a]	0.410	0.681	0.27±0.12
	Cu (PPM)	0.02±0.01[a]	0.02±0.01[a]	0.01±0.01[a]	0.600	0.579	0.02±0.01
Antinutrients (mg/100g)	**Phytates**	22.44±0.96[a]	21.40±0.53[a]	20.91±0.17[a]	4.488	0.064	21.58±0.8
	Oxalates	38.83±2.69[a]	32.52±1.02[b]	28.69±0.35[c]	28.096	**0.001**	

Per raw, values with various lower scripts are different at 5% significance. P-value, statistical probability value; F-value, statistical Ficher value; Vit C, vitamin C; P, phosphorous; K, potassium; Ca, calcium; Mg, magnesium; Na, sodium; Fe, iron; Mn, manganese; Zn, zinc; Cu, copper.

Indeed, fibers have the capacity to complex with carcinogenic molecules, thus preventing their contact with the colon and facilitating their excretion [25, 26]. They play a protective role against constipation and also against colorectal cancer. Consumption of aubergine may therefore increase gastric volume and constitute a post-ingestive state to reach a state of satiety more quickly [26, 27].

The total phenols content (55.94-66.34 mg/100g) is interesting. They are credited with many health benefits, such as reduction of risks of cardiovascular, inflammatory or neurodegenerative diseases, cancer prevention, antiplatelet effects, blood pressure regulation, etc [28].

Aubergine also contains 6.25-6.74 mg/100 mg vitamin C. Its consumption in a diversified diet added with other vegetables is highly beneficial. Indeed, vitamin C contributes in the health of bones, cartilage, teeth, and gums. It also protects against infections, accelerates healing and promotes the absorption of iron.

The study showed that Solanum aethiopicum anguivi contains numerous minerals. Generally, the markets investigated display similar mineral values. Thus, the usual availability of Solanum aethiopicum anguivi along seasons on markets in rural areas is favorable for its consumption in various diets for populations. In fact, a diet richer in calcium and phosphorus is a beneficial in the prevention of osteoporosis and also the reduction in the risks of hypertension and prostate [29]. The mineral potassium increases the cardiovascular well-being, while the magnesium is recommended for the prevention of many concerns involving myocardial infarction [30].

V. CONCLUSION

From the study, the aubergine Solanum aethiopicum anguivi could be accounted as a vegetable which consumption is very beneficial for the population in Northern Côte d'Ivoire since it's widely produced by local farmers. It is a low calory food and can be promoted in balanced diets. It also regulates the appetite thanks to the significant fiber density. S. aethiopicum anguivi contains minerals and antioxidants namely vitamin C and polyphenols, and its consumption has important roles in the prevention of cancer and other cardiovascular diseases.

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Fraxinus Angustifolia for Planting in Sardinia (Italy) A Case Study of Innovative Agroforestry System

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***Abstract*—** *The case study reports about the development of a 18 years old* Fraxinus angustifolia *Vahl. tree plantation located in a representative test site for climate, soil and land use characters, of the agricultural planes of southern Sardinia, Italy. The favourable results about diameter at breast height (DBH) and other tree variables together with high potentiality for many ecosystem services too can identify this* Fraxinus angustifolia *tree plantation case study as an innovative agroforestry system.*

Keywords*— *Agroforestry, Fraxinus angustifolia, plantation, Sardinia.

I. INTRODUCTION

The Sardinia island (Italy) has traditional agroforestry systems but a high potential value for innovative systems too [1-2]. The agroforestry is integration of trees, crops and livestock on the same area of land and can be applied to all agricultural systems by planting trees on agricultural land or introducing agriculture in existing woodland [3-4]. The good practices in different situations assure many ecosystem services (soil protection, reduction of diffuse pollution, carbon sequestration, climate mitigation and to combat desertification, biodiversity and landscape conservation) too [5-6-7-8]. The case study reports about the development of an 18 years old *Fraxinus angustifolia* Vahl. [9] tree plantation located in a representative test site for climate, soil and land use characters, of the agricultural planes of southern Sardinia, Italy (Fig.1), looked as innovative agroforestry system.

II. MATERIAL AND METHOD

The case study concerns a plantation of *Fraxinus angustifolia* Vahl. established during the autumn of 1999 year at the Sanluri countryside (southern Sardinia, Italy), 50 km north of Cagliari (39° 31' N, 3° 36' E), partially covered by mediterranean vegetation dominated by *Cistus* spp., in the climate of western Mediterranean region (*semiarid type of tepido Mediterranean sub-climate*) with 610 mm of average annual rainfall, 15,6 C° of average annual temperature and summer drought period of 4 months (data collected from a nearby station in the decade of planting) [10-11-12]. After the harvesting of natural vegetation and an intensive preparation of soil (*typic xerocrepts*), deep over 80 cm [12], with fertilization too (kg ha^{-1} 100 P2O + 100 K2O) , the plants (2+0 bare rooted seedlings) were placed with spacing 3 x 3 m (between and within rows respectively) and density of 1111 p ha^{-1} above 6 hectares without irrigation. The observations on the survival and some phenotypic traits [13-14] of all living trees (Fig. 2-3) after 18 years (only a selective thinning occurred on 2013 year) were collected with a plot regarding over 1 hectare as total (transect of 90 x 117 m). The data have been interpreted by statistic analysis (ANOVA) and were compared with as much as reported on literature [15-16-17-18].

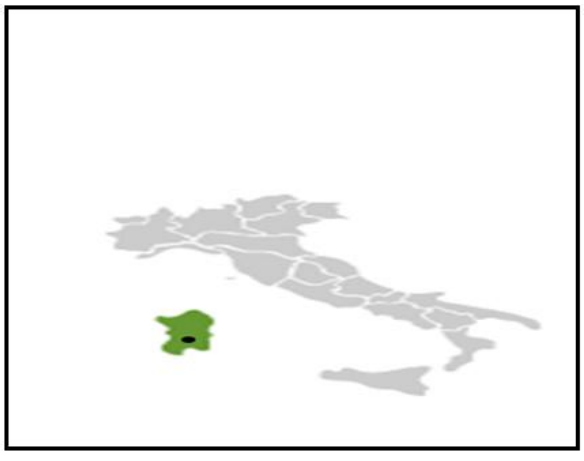

FIG.1 *Fraxinus angustifolia* tree plantation location (southern Sardinia, Italy)

FIG.2 *Fraxinus angustifolia* living trees in the plot on 2017 winter time

FIG.3 *Fraxinus angustifolia* living tree in the plot on 2017 winter time

III. RESULTS

The results, regarding the survival and some phenotypic traits of all living trees in the plot after 18 years are reported in the Figure 4, Figure 5 and in Table 1. The survival rate was almost 70 %, the average value of diameter at breast height (DBH) was 11, 9 cm, the average value of total height (H) was 8,7 m and the basal area (G ha^{-1}) was 8,35 m^2. The plot doesn't reflect significant differences regarding to each parameters.

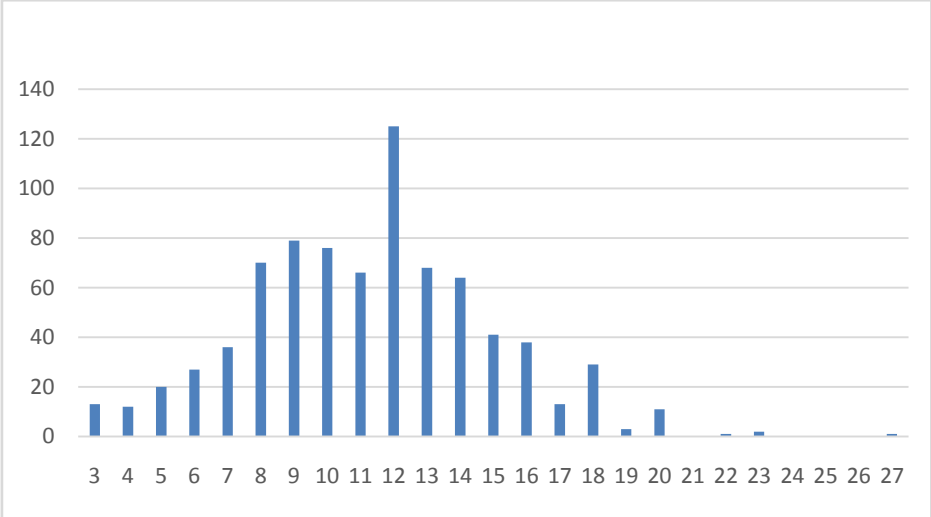

FIG. 4 Diameters (cm) distribution in the plot

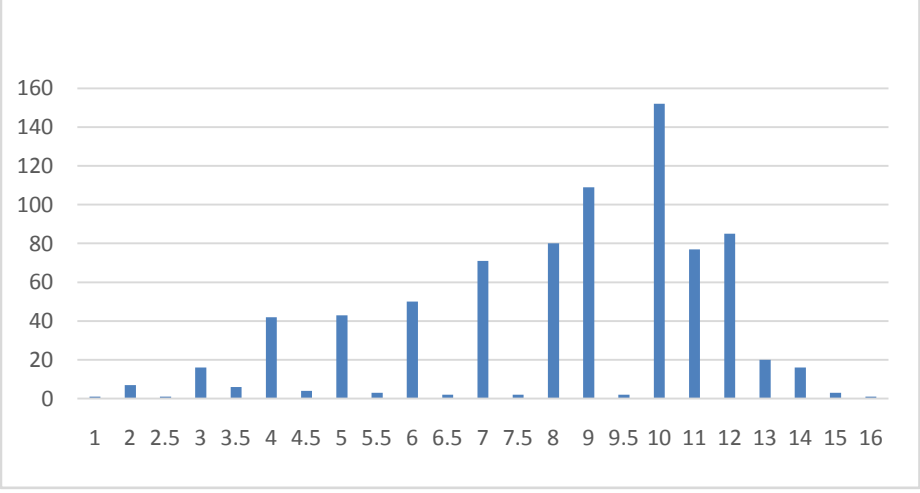

FIG. 5 Heights (m) distribution in the plot

TABLE 1
AVERAGE VALUES OF SURVEYED PARAMETERS

Average values of surveyed parameters			
Density (p ha^{-1})	DBH (cm)	H (m)	G ha^{-1} (m^2)
755	11,9	8,7	8,35

IV. DISCUSSION AND CONCLUSION

The development of a *Fraxinus angustifolia* tree plantation in a large and homogeneous agricultural area, representative as a test site for climate, soil and land use characters of the southern Sardinia (Italy) agricultural planes, after 18 years shows favourable results [15-16-17-18] about diameter at breast height (DBH), total height (H) and basal area (G ha^{-1}). The *Fraxinus angustifolia* tree plantation can be really an innovative agroforestry system in Sardinia (Italy). As matter of fact the role assigned to *Fraxinus* tree planting as in the case study at the same time appears to have high potentiality for many ecosystem services (soil protection, reduction of diffuse pollution, carbon sequestration, climate mitigation and to combat desertification, biodiversity and landscape conservation) too [5-6-7-8].

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Study of Physiochemical and Properties of CNSL based Termiticides

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Abstract— *Cashew nut shell liquid based termiticides using neem seed oil, karanj seed oil and bhilawan shell liquid, were developed and the effect of formulations on acid value, Iodine value, saponification value and unsaponifiable matter of CNSL termiticides was studied. It was observed that CNSL based termiticides were oil based therefore the acid value, Iodine value, saponification value, unsaponifiable matter, flash point, fire point, refractive index, specific gravity, viscosity and colour properties of CNSL, Neem seed oil, Karanj seed oil and Bhilawan shell liquid were reflected in the termiticide formulations with respect to the temperature.*

Keywords— termiticide, CNSL, Cashew nut.

I. INTRODUCTION

Termites are now a day becoming nuisance to urban as well as rural areas causing loss of crores of rupee world over. Hazardous and costly chemicals are being used to control the termites which are leading to dreadful diseases. Therefore, Termiticides are applied to wood furniture and other articles made of wood by brush and by dipping in solutions. Termiticide solutions are absorbed in upper layer of wood articles but may be affected by temperature and humidity. The influence of atmospheric temperature on physicochemical properties of oils may affect the mixing of oils, forming uniform coat on the surface of wood samples and absorption of solution by wood samples when dipped in it. Chemical properties of these termiticides may be responsible for the retaining of termiticide inside the wood articles and become resistant to termites. the study on '**Development of Cashew Nut Shell Liquid based Termiticide'** was undertaken in the Department of Agricultural Process Engineering, College of Agricultural Engineering and Technology, Dr. BSKKV, Dapoli with the objective to study the effect of temperature on some physicochemical properties of Cashew Nut Shell Liquid, Neem seed oil, Karanj oil and Bhilawan Shell Liquid.

II. MATERIALS AND METHODS

Cashew nut shell liquid based termiticides were made by using CNSL, Neem seed oil, Karanj seed oil and Bhilawan shell liquid in different combinations as shown in Table 1.Acid value, Iodine value, saponification value and unsaponifiable matter, flash point, fire point, refractive index, specific gravity, viscosity and colour properties of sixteen termiticides (TO_1 to TO_{16}) were determined by following standard procedure given in Table 2 and effect of formulation was studied in the field testing to know the efficacy of formulation as termiticide. The laboratory analysis was carried out in the NATP Laboratory in the College of Agricultural Engineering and Technology and Dept. of Agricultural Chemistry and Soil Science, Dr. BSKKV, Dapoli, Babasaheb Ambedkar Technical University, Lonare, Annasaheb Shinde College of Agricultural Engineering and Technology, MPKV, Rahuri and Insta, PolluTech Lab, Pune.

TABLE 1
TREATMENTS OF OIL FORMULATED CNSL BASED TERMITICIDE

No.	Treatment	Oils			
		CNSL (%)	NSO (%)	KSO (%)	BSL (%)
1	TO_1	100	0	0	0
2	TO_2	0	100	0	0
3	TO_3	0	0	100	0
4	TO_4	0	0	0	100
5	TO_5	80	10	10	0
6	TO_6	80	10	0	10
7	TO_7	80	0	10	10
8	TO_8	70	15	15	0
9	TO_9	70	15	0	15
10	TO_{10}	70	0	15	15
11	TO_{11}	60	20	20	0
12	TO_{12}	60	20	0	20
13	TO_{13}	60	0	20	20
14	TO_{14}	50	25	25	0
15	TO_{15}	50	25	0	25
16	TO_{16}	50	0	25	25

(TO- Treatment of oil formulation, CNSL- Cashew nut shell liquid, NSO- Neem seed oil, KSO- Karanj seed oil, BSL- Bhilawan shell liquid)

2.1 Acid Value

Acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids, normally formed during decomposition of oil glycerides, which has an adverse effect on the quality of many lipids. The value is also expressed as percent of free fatty acids calculated as oleic acid. Acid value of oil formulated termiticides was determined as per 'ISO 660: 1996'.

2.2 Iodine Value

The Iodine value of an oil/fat is the number of grams of iodine absorbed by 100g of oil/fat. The Iodine value of oil formulated termiticides was determined by using Wijs solution as per the 'AOAC 2000'.

2.3 Saponification Value

The saponification value is the quantity of potassium hydroxide (KOH) required saponifying 1g of oil. Saponification value of oil formulated termiticides was determined as per the 'AOAC, 2000'.

2.4 Unsaponifiable Matter

The unsaponifiable matter is defined as the substances soluble in oil which after saponification are insoluble in water but soluble in the solvent used for the determination. Unsaponifiable matter of oil formulated termiticides was determined as per the 'AOAC 2000'.

2.5 Flash point

Flash point is the temperature at which the sample will flash when a test flame is applied under the conditions specified for the open cup Cleveland test. It is taken as the lowest temperature at which the application of the test flame causes the vapour above the sample to ignite momentarily. Flash point of oil formulated termiticides was determined as per the 'I.S. 1448–1970' except that of BSL which was determined as per 'IS 1448 (P: 21) 1992'(Fig. 1)

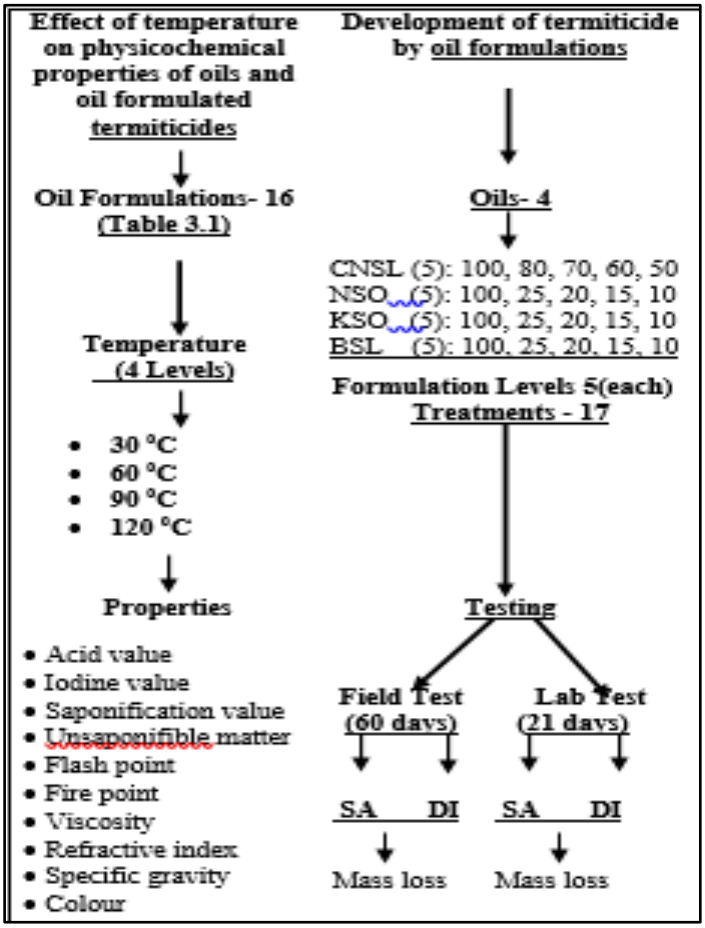

FIG. 1. Experimental design.

(FT – Field Test, LT – Laboratory Test, SA – Surface application, DI – Dipping method)

TABLE 2
METHODS FOR DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF OIL FORMULATED TERMITICIDES.

Sr. No.	Property	Method
1	Acid Value	ISO 660:1996
2	Iodine Value	AOAC, 2000
3	Saponification Value	AOAC, 2000
4	Unsaponifiable Matter	AOAC, 2000
5	Flash Point	IS 1448 – 1970, IS 1448 (P:21) 1992
6	Fire Point	IS1448 – 1970, IS 1448 (P:69) 1969
7	Viscosity	ISO 2555:1989
8	Refractive Index	AOAC, 2000
9	Specific Gravity	AOAC, 2000
10	Colour	I.S. 548(Part 1) - 1964

2.6 Fire point

The fire point is the lowest temperature at which the application of test flame causes the material to ignite and burn at least for 5 seconds under specified conditions of the test. After flash point, heating should be continued at such a rate that the increase in temperature recorded by the thermometer is neither less than 5 degrees Celsius nor more than 6 degrees Celsius per minute. The test flame was lighted and adjusted so that it is of the size of a bead 4 mm in diameter. Fire point of oil formulated termiticides was determined as per the 'I.S. 1448–1970' and fire point of BSL was determined as per 'IS 1448 (P:69) 1969'(Fig. 2b).

2.7 Viscosity

Viscosity is a measure of resistance to flow of a fluid. Although molecules of a fluid are in constant random motion, the velocity in a particular direction is zero unless some force is applied to cause fluid to flow. The magnitude of the force needed to induce flow at a certain velocity is related to the viscosity of a fluid. Viscosity of oil formulated termiticides was determined at different temperatures (30, 60, 90 and 120^{0}C) by using Brookfield Viscometer as per the 'ISO 2555:1989' (Fig. 3)

a. Flash Point **b. Fire Point**

FIG. 2: Flash point and fire point Testing (Open cup Cleveland Test).

FIG. 3: Brookfield Viscometer

2.8 Refractive index

The ratio of velocity of light in vacuum to the velocity of light in the oil or fat; more generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction, when a ray of light of known wave length (usually 589.3 nm, the mean of D lines of sodium) passes from air into the oil or fat. Refractive index of oil formulated termiticides was determined at different temperatures (30, 60, 90 and 120 ^{0}C) as per the 'AOAC 2000' by using ATAGO Refractometer (Fig. 4).

FIG. 4: Refractometer

2.9 Specific gravity

Specific gravity is the ratio of weight of oil at 30 ^{0}C to the weight of water at 30 ^{0}C. Specific gravity of oil formulated termiticides was determined by the formula given below

$$\text{Specific gravity of oil at } 30\ ^{0}\text{C} = \frac{A-B}{C-D} \quad (1)$$

Where,

A = weight in gm of specific gravity bottle with oil at 30 ^{0}C

B = weight in gm of specific gravity bottle at 30 ^{0}C

C = weight in gm of specific gravity bottle with water at 30 ^{0}C

Specific gravity of oils was determined at different temperatures (30, 60 and 90 ^{0}C) as per the 'AOAC, 2000.

2.10 Colour

The colour (L*, a* and b* values) of oil formulated termiticides was determined by using Colour Flex Meter. L* indicates the lightness and extends from 0 (black) to 100(white). The other two coordinates a* and b* indicate redness (+a) to greenness (-a) and yellowness (+b) to blueness (-b), respectively. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the specimen oil sample. Colour of oils and oil formulated termiticides was determined by Colour Flex Meter as per the 'I.S. 548(part 1)-1964'. The colour values of oil formulated termiticides were determined at 30, 60, 90 and 120 ^{0}C temperatures.

III. RESULTS AND DISCUSSION

3.1 Acid Value

Table 3 shows that acid values of CNSL (TO_1), NSO (TO_2), KSO (TO_3) and BSL (TO_4) were found 98.20, 5.90, 21.90 and 20.80 mg of KOH/g of oil, respectively. The acid value of CNSL was found the highest and that of NSO the lowest among four pureoils. IS:840-(1986), Djibril *et al*. (2015), Bobade and Khyade (2012) and Chopra and Chopra (1956) have observed the similar results of acid value of CNSL. NSO, KSO and BSL, respectively. Acid value of oils was reflected in the formulations of termiticides (TO_5 to TO_{16}) and observed in the range of 50.70 to 80.76 mg of KOH/g of oil. The acid value of termiticide TO_7 was the highest i.e. 82.76 mg of KOH/g of oil and that of TO_{14} it was 51 mg of KOH/g of oil among all termiticides. It is a relative measure of rancidity as free fatty acids, are normally formed during decomposition of oil glycerides, which is observed as acid value in mg of KOH/g of oil.

TABLE 3
ACID AND IODINE VALUE OF OIL FORMULATED TERMITICIDES.

Sr. No.	Treatment	Formulation CNSL:NSO:KSO:BSL	Acid Value (mg of KOH/g of oil)	Iodine Value mg of Iodine/100 g of oil
1	TO_1	100:0:0:0	98.20	266.70
2	TO_2	0:100:0:0	5.90	81.28
3	TO_3	0:0:100:0	21.90	85.09
4	TO_4	0:0:0:100	20.80	121.92
5	TO_5	80:10:10:0	81.36	229.30
6	TO_6	80:10:0:10	81.16	232.80
7	TO_7	80:0:10:10	82.76	233.30
8	TO_8	70:15:15:0	72.67	210.70
9	TO_9	70:15:0:15	72.64	216.70
10	TO_{10}	70:0:15:15	74.74	216.70
11	TO_{11}	60:20:20:0	64.52	193.00
12	TO_{12}	60:20:0:20	64.32	200.00
13	TO_{13}	60:0:20:20	67.52	201.00
14	TO_{14}	50:25:25:0	51.00	175.50
15	TO_{15}	50:25:0:25	50.70	180.50
16	TO_{16}	50:0:25:25	54.70	182.50

(TO-Oil Formulation Treatment, CNSL- Cashew nut shell liquid, NSO- Neem seed oil, KSO- Karanj seed oil, BSL- Bhilawan shell liquid)

3.2 Iodine Value

Table 3 shows that Iodine value of CNSL (TO_1), NSO (TO_2), KSO (TO_3) and BSL (TO_4) were found 266.70, 81.28, 85.09 and 121.92 mg of Iodine/100 g of oil, respectively. The Iodine value of CNSL was found the highest and that of NSO the lowest among the four pure oils. Asogwa *et al*. (2007), Djibril *et al*. (2015), Bobade and Khyade (2012) and Chopra and Chopra (1956) have also observed the similar results of Iodine value of CNSL. NSO, KSO and BSL, respectively. Iodine value of oils was reflected in the formulations of termiticides (TO_5 to TO_{16}) and observed in the range of 175.50 to 233.30 mg of Iodine/100 g of oil. Iodine value of termiticideTO_7 was the highest 233.3 mg of Iodine/100 g of oil and that of TO_{14} the lowest i.e. 175.5 mg of Iodine/100 g of oil among all oil formulated termiticides.

3.3 Saponification Value

Table 4 shows that the saponification value of CNSL (TO_1), NSO (TO_2), KSO (TO_3) and BSL (TO_4) was 123.20, 156.80, 184.80 and 196.00 mg of KOH/g oil, respectively. It was found the highest in BSL and in CNSL the lowest among four pure oils. Mukhopadhyaya*et al*. (2010), Djibril et al. (2015), Bobade and Khyade (2012) and Chopra and Chopra (1956) have also observed the similar results of saponification value of CNSL. NSO, KSO and BSL, respectively. The saponification value of the oils was reflected in the formulations of termiticides(TO_5 to TO_{16}) and observed in the range of 133 to 196 mg of KOH/g oil. The saponification value of formulation TO_5 was observed the lowest i.e.133mg of KOH/g oil and that of TO_{15} the highest i.e.196 mg of KOH/g oil, among all oil formulated termiticides.

3.4 Unsaponifiable Matter

Table 4 shows that the unsaponifiable matter in the CNSL (TO_1), NSO (TO_2), KSO (TO_3) and BSL (TO_4) was 1.036, 1.285, 2.885 and 6.36 percent, respectively. It was found the highest in BSL (TO_4) i.e. 6.36 per cent and that of the CNSL (TO_1) the lowest among four oil formulations (TO_1 to TO_4) (pure oils). Prasad (2014), Puri (1999), Bobade and Khyade (2012), Chopra and Chopra (1956) have also observed the similar results of unsaponifiable matter in CNSL NSO, KSO and BSL, respectively. The unsaponifiable matter in oils was reflected in formulations of termiticides(TO_5 to TO_{16}) and observed in the range of 0.417 to 2.311 percent. The unsaponifiable matter in oil formulation TO_5 was the lowest i.e. 0.417 percent and that in TO_{16} it was the highest i.e. 2.311 percent among all formulations.

TABLE 4
SAPONIFICATION VALUE OF OIL FORMULATED TERMITICIDES.

Sr. No.	Treatment	Formulation CNSL:NSO:KSO:BSL	Saponification Value (mg of KOH/g of oil)	Unsaponifiable Matter (%)
1	TO_1	100:0:0:0	123	1.036
2	TO_2	0:100:0:0	157	1.285
3	TO_3	0:0:100:0	185	2.885
4	TO_4	0:0:0:100	196	6.36
5	TO_5	80:10:10:0	133	0.417
6	TO_6	80:10:0:10	134	0.765
7	TO_7	80:0:10:10	137	0.925
8	TO_8	70:15:15:0	137	0.625
9	TO_9	70:15:0:15	139	1.147
10	TO_{10}	70:0:15:15	143	1.387
11	TO_{11}	60:20:20:0	142	0.814
12	TO_{12}	60:20:0:20	144	1.529
13	TO_{13}	60:0:20:20	150	1.829
14	TO_{14}	50:25:25:0	147	1.042
15	TO_{15}	50:25:0:25	196	1.911
16	TO_{16}	50:0:25:25	157	2.311

(TO-Oil Formulation Treatment, CNSL- Cashew nut shell liquid, NSO- Neem seed oil, KSO- Karanj seed oil, BSL- Bhilawan shell liquid)

3.5 Flash point

Table 5 shows that flash point of CNSL (TO_1), NSO (TO_2), KSO (TO_3) and BSL (TO_4) were 216, 174, 224 and 269^{0}C, respectively. The flash point of BSL was the highest (269 ^{0}C) and that of NSO the lowest (174 ^{0}C) among four pure oils. Prasad (2014[c]), Djibril *et al*. (2015[e]), Bobade and Khyade (2012[g]), have reorted the similar results of flash point of CNSL, NSO and KSO, respectively. It was observed that BSL was not tried for its usefulness in fuel hence no reference was found for flash point. The flash point of oils was reflected in termiticides (TO_5 to TO_{16}) and observed in the range of 188 to 220 ^{0}C. Flash point of termiticide TO_8 was found the highest (220 ^{0}C) and that of T_{14} the lowest (188 ^{0}C) among all the termiticides. Flash point indicates the sensitivity of formulation at high temperature to ignite during handling and storage. Flash point of all the termiticides was in the range of 174 to 269 ^{0}C i.e. at quite safe level.

3.6 Fire point

Table 5 shows that fire point of CNSL (TO_1), NSO (TO_2), KSO (TO_3) and BSL (TO) be 221, 181, 229 and 280^{0}C, respectively. The fire point of BSL was the highest and that of NSO the lowest among four pure oils. Prasad (2014[d]), Djibril *et al*. (2015[f]), Bobade and Khyade (2012[h]), have reported similar results of fire point in CNSL, NSO and KSO, respectively. It was observed that BSL was not tried for its usefulness in fuel hence no reference was found for fire point. The fire point of oils was reflected in termiticides (TO_5 to TO_{16}) and observed in the range of 195 to 236^{0}C. Fire point of termiticide TO_{10} was found the highest (236^{0}C) and that of TO_{14} the lowest (195^{0}C) among all the oil formulated termiticides. Fire point indicates the sensitivity of formulation at high temperature to catch fire in the handling and storage. Flash point of all the formulations was in the range of 181 to 280^{0}C i.e. at quite safe level.

TABLE 5
FLASH POINT AND FIRE POINT OF OIL FORMULATED TERMITICIDES.

Sr. No.	Treatment	Formulation CNSL : NO : KO : BSL	Flash Point (^{0}C)	Fire Point (^{0}C)
1	TO_1	100:0:0:0	216	221
2	TO_2	0:100:0:0	174	181
3	TO_3	0:0:100:0	224	229
4	TO_4	0:0:0:100	269	280
5	TO_5	80:10:10:0	204	211
6	TO_6	80:10:0:10	198	204
7	TO_7	80:0:10:10	206	229
8	TO_8	70:15:15:0	220	226
9	TO_9	70:15:0:15	202	233
10	TO_{10}	70:0:15:15	195	236
11	TO_{11}	60:20:20:0	205	208
12	TO_{12}	60:20:0:20	203	220
13	TO_{13}	60:0:20:20	200	214
14	TO_{14}	50:25:25:0	188	195
15	TO_{15}	50:25:0:25	190	197
16	TO_{16}	50:0:25:25	197	200

(TO - Oil formulation treatment, CNSL- Cashew nut shell liquid, NSO - Neem seed oil, KSO - Karanj seed oil, BSL- Bhilawan shell liquid)

3.7 Viscosity

Table 6 shows that viscosity of CNSL (TO_1), NSO (TO_2), KSO (TO_3)and BSL (TO_4) was observed 562, 89, 32 and 1070 cP, at 30 ^{0}C, respectively and was found to be decreased to 395, 5, 4 and 422 with increase in temperature from 30 to 120 ^{0}C. The viscosity of BSL was found the highest and that of KSO the lowest among four pure oils. Viscosity of NSO was found to be decreased 94.38% with increase in the temperature from 30 to 120 ^{0}C. Asogwa *et al*. (2007[c]), Djibril *et al*. (2015[h]), Bobade and Khyade (2012[i]), and Lad *et al*. (2016[b]) have also observed the similar results of viscosity of CNSL. NSO, KSO and BSL, respectively. The viscosity of oils was reflected in termiticides (TO_5 to TO_{16}) and observed in the range of 296 to 1070 cP in the temperature range of 30 to 120 ^{0}C. The viscosity of termiticide TO_{15} was the highest i.e. 838, 782, 539 and 495 cP and that of the TO_{14} the lowest i.e. 311, 293, 267 and 234 cP at 30, 60, 90 and 120 ^{0}C, respectively among oil formulated termiticides. All the oils melt at higher temperatures therefore the viscosity of oil formulated termiticides was found decreased with increasing temperature from 30 to 120 ^{0}C.

TABLE 6
EFFECT OF TEMPERATURE ON VISCOSITY OF OIL FORMULATED TERMITICIDES.

Sr. No.	Treatment	Formulation CNSL:NSO:KSO:BSL	Viscosity (cP) Temperature (^{0}C)				Response to temperature (%)
			30	60	90	120	
1	TO_1	100:0:0:0	562	475	448	395	29.72
2	TO_2	0:100:0:0	89	20	10	5	94.38
3	TO_3	0:0:100:0	32	12	6	4	87.50
4	TO_4	0:0:0:100	1070	890	650	422	60.56
5	TO_5	80:10:10:0	462	449	343	321	30.52
6	TO_6	80:10:0:10	673	645	489	437	35.07
7	TO_7	80:0:10:10	667	621	467	405	39.28
8	TO_8	70:15:15:0	411	385	352	318	22.63
9	TO_9	70:15:0:15	727	679	438	410	43.60
10	TO_{10}	70:0:15:15	719	688	413	391	45.62
11	TO_{11}	60:20:20:0	361	344	310	296	18.01
12	TO_{12}	60:20:0:20	783	721	474	419	46.49
13	TO_{13}	60:0:20:20	771	705	458	401	47.99
14	TO_{14}	50:25:25:0	311	293	267	234	24.76
15	TO_{15}	50:25:0:25	838	782	539	495	40.93
16	TO_{16}	50:0:25:25	824	781	516	473	42.60

(TO - Oil formulation treatment, CNSL- Cashew nut shell liquid, NSO - Neem seed oil, KSO - Karanj seed oil, BSL- Bhilawan shell liquid)

3.8 Refractive index

Table 7 shows that refractive index of BSL (TO_4) was the highest i.e. 1.526, 1.510, 1.504 and 1.489 and that of KSO (TO_3) the lowest i.e.1.423, 1.411, 1.406 and 1.391 at the temperatures 30, 60, 90 and 120 ^{0}C, respectively among the four pure oils. Mukhopadyaya *et al*. (2010[b]), Djibril *et al*. (2015[j]), Bhalerao and Sharma (2014), and Lad *et al*. (2016) have also observed the similar results of refractive index of CNSL. NSO, KSO and BSL, respectively. Refractive index value of pure oils was found reflected in all the oil formulated termiticides. Among termiticides the highest refractive index was recorded in TO_6, i.e. 1.510, 1.504, 1.499 and 1.469 and the lowest in the termiticide TO_{14} i.e. 1.479, 1.472, 1.465 and 1.434, at 30, 60, 90 and 120 ^{0}C temperatures, respectively. The refractive index values of all the oil formulated termiticides were found the highest at 30 ^{0}C and then decreased with increase in the temperature from 30 to 120 ^{0}C.

3.9 Specific gravity

Table 8 shows that the specific gravity values 0.9867, 0.9848 and 0.9828 of BSL(TO_4) were the highest and that of KSO (TO_3) the lowest i.e. 0.9325, 0.9324 and 0.9322, at the temperatures 30, 60 and 90 ^{0}C, respectively among the four oils. Asogwa *et al*. (2007[d]), Djibril *et al*. (2015[k]), Bobade and Khyade (2012[k]), and Chopra and Chopra (1956[f]) have also observed the similar results of viscosity of CNSL. NSO, KSO and BSL, respectively. Among oil formulated termiticides from TO_5 to TO_{16}, the specific gravity values of the TO_{15} were found the highest i.e. 0.9657, 0.9485, and 0.9347; and that of TO_{14} the lowest i.e. 0.9428, 0.9232 and 0.9077 at the temperatures 30, 60 and 90 ^{0}C, respectively. The specific gravity of all the oil formulated termiticides was found decreased with increase in the temperature from 30 to 90 ^{0}C. With increase in temperature the molecules of oil formulated termiticides melted due to which its specific gravity was decreased.

TABLE 7
EFFECT OF TEMPERATURE ON REFRACTIVE INDEX OF OIL FORMULATED TERMITICIDES.

Sr. No.	Treatment	Formulation	Refractive Index				Response to temperature (%)
			Temperatures (°C)				
		CNSL : NO : KO : BSL	30	60	90	120	
1	TO_1	100:0:0:0	1.512	1.508	1.486	1.471	2.71
2	TO_2	0:100:0:0	1.471	1.462	1.448	1.443	1.90
3	TO_3	0:0:100:0	1.423	1.411	1.406	1.391	2.25
4	TO_4	0:0:0:100	1.526	1.510	1.504	1.489	2.42
5	TO_5	80:10:10:0	1.499	1.494	1.49	1.459	2.67
6	TO_6	80:10:0:10	1.510	1.504	1.499	1.469	2.72
7	TO_7	80:0:10:10	1.505	1.499	1.495	1.464	2.72
8	TO_8	70:15:15:0	1.491	1.486	1.481	1.453	2.55
9	TO_9	70:15:0:15	1.507	1.501	1.495	1.468	2.59
10	TO_{10}	70:0:15:15	1.500	1.494	1.489	1.46	2.67
11	TO_{11}	60:20:20:0	1.496	1.479	1.473	1.448	3.21
12	TO_{12}	60:20:0:20	1.506	1.499	1.493	1.467	2.59
13	TO_{13}	60:0:20:20	1.507	1.489	1.485	1.457	3.32
14	TO_{14}	50:25:25:0	1.479	1.472	1.465	1.814	3.04
15	TO_{15}	50:25:0:25	1.505	1.497	1.49	1.467	2.52
16	TO_{16}	50:0:25:25	1.492	1.483	1.479	1.454	2.55

(TO - Oil formulation treatment, CNSL- Cashew nut shell liquid, NSO - Neem seed oil, KSO- Karanj seed oil, BSL- Bhilawan shell liquid)

3.10 Colour

Table 9 shows that the DL*(+ whiteness and –blackness) values of all the oils (TO_1 to TO_4) and oil formulated termiticides (TO_5 to TO_{16}) were negative showing colour darkness (blackness). The oil KSO (TO_3) was having the least darkness values (-63.15) among all the oil formulated termiticides and it was seconded by NSO (TO_2) (-71.26) at 30 ^{0}C. DL* value was decreased in KSO (TO_3) initially when heated from 30 to 90 ^{0}Cand then found increased at 120 ^{0}C. Darkness values of TO_1, TO_2 and TO_4 were increased with increase in temperature from 30 to 120 ^{0}C. The Da*(+ redness and – greenness) value of CNSL, NSO and BSL and all oil formulations was less green whereas that of KSO it was 11.65 i.e. reddish. The Da*(+ redness and – greenness) value of CNSL was observed decreased, of NSO increased and of BSL slightly increased where as that of KSO it was increased with increase in temperature from 30 to 120 ^{0}C.

The Db* (+yellowness and – blueness) value of CNSL, NSO and BSL and all oil formulated termiticides was bluish whereas that of KSO it was (11.06) yellowish. With increase in temperature from 30 to 120 ^{0}C, the yellowness of KSO was increased (from 11.06 to 19.68), blueness of NSO slightly increased and that of CNSL and BSL blueness was increased. The reflection of colour values of oils is clearly observed in all the oil formulated termiticides with slight differences at the temperatures from 30 to 120 ^{0}C. Asogwa et al. (2007[e]), Djibril et al. (2015[l]), Bobade and Khyade (2012[l]), and Chopra and Chopra (1956[g]) have also observed the similar results of colour values of CNSL, NSO, KSO and BSL, respectively.

TABLE 8
EFFECT OF TEMPERATURE ON SPECIFIC GRAVITY OF OIL FORMULATED TERMITICIDES.

Sr. No.	Treatment	Formulation	Specific Gravity			Response to temperature (%)
			Temperature (°C)			
		CNSL : NO : KO : BSL	30	60	90	
1	TO_1	100:0:0:0	0.948	0.942	0.939	0.94
2	TO_2	0:100:0:0	0.938	0.937	0.933	0.50
3	TO_3	0:0:100:0	0.933	0.932	0.932	0.03
4	TO_4	0:0:0:100	0.987	0.985	0.983	0.40
5	TO_5	80:10:10:0	0.950	0.940	0.930	1.54
6	TO_6	80:10:0:10	0.960	0.950	0.930	3.43
7	TO_7	80:0:10:10	0.950	0.930	0.910	3.51
8	TO_8	70:15:15:0	0.950	0.940	0.920	2.91
9	TO_9	70:15:0:15	0.960	0.940	0.940	2.45
10	TO_{10}	70:0:15:15	0.950	0.940	0.92	3.78
11	TO_{11}	60:20:20:0	0.950	0.930	0.91	3.51
12	TO_{12}	60:20:0:20	0.950	0.940	0.920	3.40
13	TO_{13}	60:0:20:20	0.950	0.940	0.910	4.88
14	TO_{14}	50:25:25:0	0.940	0.920	0.910	3.72
15	TO_{15}	50:25:0:25	0.970	0.950	0.930	3.21
16	TO_{16}	50:0:25:25	0.960	0.950	0.930	3.95

(TO - Oil formulation treatment, CNSL- Cashew nut shell liquid, NSO- Neem seed oil, KSO- Karanj seed oil, BSL- Bhilawan shell liquid)

TABLE 9
EFFECT OF TEMPERATURE ON COLOUR OF OILS AND OIL FORMULATED CNSL BASED TERMITICIDES.

Treatment	Formulation CNSL:NSO: KSO:BSL	Colour difference values at 30 ^{0}C			Colour difference values at 60 ^{0}C			Colour difference values at 90 ^{0}C			Colour difference values at 120 ^{0}C		
		DL*	Da*	Db*	DL*	Da*	Db*	DL*	Da*	Db*	DL*	Da*	Db*
TO_1	100:0:0:0	-72.49	0.85	-2.26	-72.40	0.72	-2.15	-72.59	0.52	-2.18	-73.14	0.91	-2.59
TO_2	0:100:0:0	-71.26	1.48	0.08	-71.34	2.52	0.15	-71.50	1.74	-0.12	-72.53	5.70	1.11
TO_3	0:0:100:0	-63.15	11.65	13.06	-60.67	10.13	17.01	-58.74	9.44	19.68	-68.61	10.75	13.47
TO_4	0:0:0:100	-72.36	0.44	-2.35	-72.79	0.46	-2.31	-72.53	0.49	-2.35	-73.09	0.59	-2.57
TO_5	80:10:10:0	-73.36	1.13	-1.94	-72.93	1.19	-2.06	-73.52	1.48	-2.04	-73.25	1.58	-2.01
TO_6	80:10:0:10	-73.39	0.84	-1.76	-73.38	0.69	-1.34	-73.35	0.68	-1.75	-73.40	1.43	-2.46
TO_7	80:0:10:10	-72.94	1.47	-2.10	-72.95	1.27	-2.26	-72.73	1.91	-2.50	-72.46	1.81	-2.70
TO_8	70:15:15:0	-72.29	0.48	-2.55	-72.42	0.45	-2.88	-72.43	0.29	-2.93	-72.60	0.86	-3.16
TO_9	70:15:0:15	-71.99	0.80	-3.33	-72.19	0.81	-3.11	-72.17	0.59	-2.80	-72.29	0.61	-2.88
TO_{10}	70:0:15:15	-72.14	0.86	-2.88	-72.03	0.61	-2.94	-72.26	0.58	-3.10	-72.19	0.35	-2.99
TO_{11}	60:20:20:0	-71.46	0.50	-2.73	-71.72	0.70	-2.79	-71.83	0.54	-2.80	-71.65	0.66	-2.50
TO_{12}	60:20:0:20	-72.02	0.66	-3.04	-72.62	0.56	-2.98	-72.19	-0.10	-2.70	-72.36	0.76	-2.97
TO_{13}	60:0:20:20	-72.15	1.13	-3.37	-72.35	0.77	-3.33	-72.34	1.09	-2.85	-72.39	0.63	-2.60
TO_{14}	50:25:25:0	-72.06	0.33	-2.56	-72.22	0.28	-2.94	-72.31	1.06	-3.13	-72.13	0.60	-2.83
TO_{15}	50:25:0:25	-72.48	0.90	-2.91	-72.27	0.27	-2.50	-72.70	0.46	-2.94	-72.76	0.40	-2.71
TO_{16}	50:0:25:25	-72.13	0.96	-3.11	-72.42	1.17	-3.07	-72.69	0.67	-3.42	-72.65	0.63	-3.10
+DL* = Whiteness, –DL*= Blackness, +Da*= Redness, -Da*= Greenness, +Db* = Yellowness, -Db*= Blueness													

(TO - Oil formulation treatment, CNSL- Cashew nut shell liquid, NSO- Neem seed oil, KSO- Karanj seed oil, BSL- Bhilawan shell liquid)

From the results it is observed that properties of CNSL, NSO, KSO and BSL were found reflected in all the CNSL based termiticides with respect to the proportions of oils in the formulations.

IV. SUMMARY AND CONCLUSION

To search the alternatives to the chemical termiticides, CNSL based termiticides were developed by using NSO, KSO and BSL in different proportions. Physicochemical properties of sixteen formulations were determined. Effect of temperature on some properties as well as effect of proportions of oils on physicochemical properties of sixteen formulations of termiticide was studied. From the results obtained it is concluded that physicochemical properties of CNSL, NSO, KSO and BSL can be reflected in the CNSL based termiticides developed.

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Enhancement of β-1,3 Glucanase Production from *Penicillium oxalicum* T3.3

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Abstract *— β-1,3 glucanases are semi-constitutive hydrolytic enzymes that can degrade glucan molecules embedded in the cell wall components of cereals and some species of fungi resulted in production of D-glucose. This enzyme has a great potential and interest in biotechnology, agricultural and also industrial field. However, there is little reports on the production of β-1,3 glucanase by Penicillium oxalicum. Therefore, the cultural conditions which stimulate in vitro production of β-1,3 glucanase enzyme by P. oxalicum T3.3 and characterization of β-1,3 glucanase enzyme activity were determined.Various parameters such as different types of carbon and nitrogen sources, initial pH medium, agitation speed and surfactants were investigated. The optimization was carried out by varying and optimizing one variable at a time. The highest production of β-1,3 glucanase activity of 84.73 U/mL was obtained using seaweed Undaria pinnatifida as substrate at concentration of 1% (w/v), peptone and yeast extract as nitrogen source at 0.3% and 0.2% respectively, initial medium pH 5, agitation speed at 200 rpm and with addition of sodium dodecyl sulfate as surfactant. Under these conditions, β-1,3 glucanase activity increased by 38.6%. Enzyme characterization was also performed which indicated that this enzyme is thermostable and showed optimum activity at 50°C, pH 5 and can retained its activity around 80% up to 4 h at this condition.The optimization of β-1,3 glucanase production by P.oxalicum required adjustment of different types of carbon and nitrogen sources, initial pH medium, agitation speed and surfactants. This enzyme characterization has revealed its great potential towards detergent, beer and food fermentation industries whose manufacturing conditions are largely acidic.*

Keywords— β-1,3 glucanase, characterization, optimization, Penicillium oxalicum.

I. INTRODUCTION

β-1,3-glucanase is an important enzyme in the industrial and agricultural processing field. β-1,3 glucanase enzymes have received attention in many fields of science and biotechnology because many cultures of microorganisms widely used in industry produce β-1,3 glucanase (Kulminskaya *etal*., 2001). The resistance of this enzyme to denaturation by high temperature and pH extremes makes it particularly essential in various functions (Beshay *et al*., 2011). β-1,3-glucanases have been reported to be produced by a variety of organisms such as bacteria, fungi, and higher plants and many of them have been purified and characterized (Martin *et al*., 2007). β-1,3 glucanase can be used as biocontrol agent againstplant-pathogenic fungi and act as hydrolyticenzyme for the destruction of cell wall of fungal and structure of β-1,3-glucan (Beshay *et al*., 2003). Besides, for industrial purpose they are used commercially in combination with other enzymes in the production of beer and in the brewery, for barley-beta glucan degradation for animal feed. Furthermore, they can be used as effective additives in laundry detergents. They can also be used for saccharification of agricultural and industrial wastes to provide glucose syrups for animal use (Doughari and Hamuel 2011). *Penicillium sp* has also been reported to produce hydrolytic enzymes including chitinase and β-glucanase which are involved in the degrading of fungal cell wall (Chen *et al*., 2012; Patil *et al*., 2013). In another study, it was suggested that *P. oxalicum* secretes chitinase and β-glucanases to degrade and penetrate into the conidiophores and spores of *Nigrospora oryzae* (Sempere and Santamarina, 2008).

To the best of our knowledge there is little reports on the production of β-1,3 glucanases by *P. oxalicum.* Thus, a study on the optimization of β-1,3 glucanases production using this fungus is important to be carried out in order to enhance β-1,3 glucanases production. The most important aspects to decrease the production cost are optimization of media and process conditions (Goshal *et al.,* 2011). In this study, optimization of β-1,3 glucanases production was done by using the conventional method, which involved varying one variable at a time while the other variables were kept constant. Therefore, the objectives of this study were to determine the cultural conditions which stimulate the *in vitro* production of β-1,3 glucanase enzyme by *P. oxalicum* T3.3 and to characterize the β-1,3 glucanase enzyme activity.

II. MATERIAL AND METHOD

2.1 Microorganisms and cultivation

Penicillium oxalicum T3.3 was obtained from the Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. For maintenance, the fungus culture was grown on Potato dextrose agar (PDA) (Difco, USA) plates at 30ºC for 7 days. After 7 days of incubation, the matured spores were harvested by covering with approximately 10 mL of sterile distilled water. The spore suspensions of the seven day old colonies of the fungus were probed gently with a Pasteur pipette tip. Then, the collected spore suspension was transferred to a sterile tube. The spore suspension concentration was determined by using a haemacytometer. For long term stock cultures, 0.7 mL of the stock spore solution was resuspended with 0.3 mL of 80% sterile glycerol in aseptically condition. The stock spore solution was kept at -20ºC.

2.2 Enhancement of β-1,3 glucanase production

The enhancement of β-1,3 glucanases production by *P. oxalicum* T3.3 were studied by varying and optimizing one variable at a time using shake flasks under various culture conditions such as different types of substrates (fungal cell wall, seaweed, glucose and rice husk). A basal medium was used which composed of (g L^{-1} of distilled water) peptone, 3.0; $(NH_4)_2SO_4$, 2.0; yeast extract, 0.5; KH_2PO_4, 4.0; $CaCl_2.2H_2O$, 0.3; $MgSO_4.7H_2O$, 0.3 supplemented with substrates, 1.0; according to Cao *et al.*, (2009). A quantity of 50 mL of the basal medium was put into 150 mL Erlenmeyer flask and autoclaved at 121^{o}C, for 15 min. The different concentration (0.5%, 1.0%, 1.5%, 2.0% and 3.0%) of the best substrate (seaweed) was further investigated. Other parameters studied for enhancement of β-1,3 glucanases production including: incubation period, initial pH medium, agitation speed (at 120, 150, 180 and 200 rpm) and addition of different types of nitrogen sources (combinations of peptone, yeast extract and $(NH_4)_2SO_4$ at 3:2:2 ratio respectively while the control medium contained nitrogen sources in the basal medium before modifications at 3:0.5:2 ratio respectively) The effect of addition of surfactant (0.02% (v/v) Tween 20, Tween 80, Triton X-100 and 0.02% (w/v) sodium dodecyl sulphate) has been studied. Spores suspension inoculum of *P. oxalicum* T3.3 (1.0×10^6 spores mL^{-1} of culture medium) was inoculated into each flask. The cultures were grown at 30^{o}C for 5 days at 150 rpm. After 5 days, culture filtrates from the fermentation were filtered and centrifuged at 4^{o}C for 10 min at 4000 × g and the supernatants were assayed for enzyme activity and protein determination. All experiments were carried out in triplicates.

2.3 Assays of enzyme activities

In order to determine the fungal biomass, measurement of N-acetylglucosamine released as a result of acid hydrolysis of chitin is one of the indirect measures of fungal growth (Fang *et al.*, 2010; Babitha and Carvahlo, 2008). Chitin is a component of fungal biomass which is present in the fungal cell wall and it is used as a fungal growth indicator. Fungal biomass was expressed as milligrams per gram of substrate (original dry weight) according to the standard curve using glucosamine with a series of different concentrations (2-10 mg mL^{-1}) as a standard. The fungal biomass glucosamine was computed as the measured glucosamine minus the background glucosamine content of the substrates only.

For dry cell weight estimation, fermented substrate was oven dried at 60°C overnight. A 5 mL 72% (v/v) sulphuric acid was added to 0.4 g substrate. It was then agitated at 130 rpm, room temperature for 30 min. To this acid hydrolysate, 54 mL distilled water was added and the mixture was autoclaved at 121°C for 2 h. The pH was adjusted to pH 7 using 10 M, and 0.5 M NaOH. In a screw cap glass tube, 1 mL hydrolysed sample was mixed with 1 mL acetyl-acetone reagent and it was incubated in boiling water bath for 20 min. Then it was cooled under running tap water prior to the addition of 6 mL absolute ethanol. After it was mixed well, 1 mL Ehrlich reagent was added and the tubes were immediately lidded with the screw cap. Then the mixture was shaken vigorously and the excess CO_2 was released. The absorbance was read at the 530 nm using a spectrophotometer. One mL of sample was replaced by 1 mL distilled water and served as blank sample.

β-1,3 glucanase activity was assayed by adding 0.05 ml of the culture supernatant with 0.05 ml of 0.05 mol L^{-1} acetate buffer (pH 5.5), which contained 0.1 mg of laminarin (Sigma) at 50^{o}C for 30 min. Then, the reducing sugar concentration was examined by the method described by (Miller, 1960). One unit (U) of β-1,3 glucanase activity produced was defined as the total of enzyme that produced 1μmol of reducing sugar in 30 minutes under the above conditions (Cao *et al*., 2009). The soluble protein concentration was determined according to the method of Bradford protein assay using bovine serum albumin as the protein standard. Next, the absorbance of the supernatant was read at 595 nm.

2.4 Statistical data analysis

The data were statistically analyzed using software SAS (ver.9.3). The T tests of least significant differences (LSD) were used to compare the differences of means from triplicate experiments among treatment groups. Differences of ($P<0.05$) were considered significant.

III. RESULTS AND DISCUSSION

3.1 Screening of substrates for β-1,3 glucanase production

Different types of substrates were primarily screened for their abilities to produce β-1,3 glucanase enzyme which were fungal cell wall, seaweed, glucose and rice husk (Fig. 1). As shown in Fig.1, fungal cell wall was the best substrate and showed significantly ($p< 0.05$) higher β-1,3 glucanase compared to the other substrates. This was then followed by seaweed, glucose and rice husk. Maximum β-1,3 glucanase production was achieved on culture medium containing fungal cell wall as substrate which was 48.72 UmL^{-1}. From the screening of the substrates, medium containing fungal cell wall as substrate showed maximum β-1,3 glucanase production. Chernin and Chet, (2002) has suggested that most fungal cell walls contain chitin as the major structural backbone organized in regularly ordered layers and filled with β-1,3 glucan arranged in an amorphous manner. Thus, β-1,3 glucanase enzyme will hydrolyzes glucan molecules leading to the production of D-glucose, which serves as a carbon source (Tang-Yao, 2002). But in this study, fungal cell wall preparation was time consuming and laborious because it needed to undergo the fermentation process. Therefore, seaweed was chosen as the best substrates for the production of β-1,3 glucanase as it is can be used readily and is relatively cheap. On the other hand, laminarin is one of the main structural components of *Undaria pinnatifida* seaweed (Tang *et al*., 2009). It will be also hydrolyzed by β-1,3 glucanase leading to the production of glucose. Thus, these observations have shown that seaweed also has a great potential as the substrate for β-1,3 glucanase production.

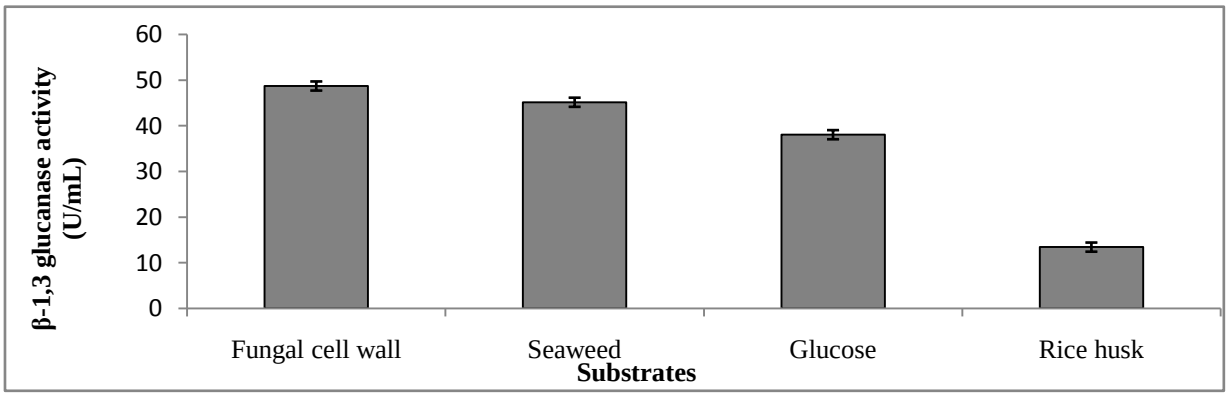

FIG. 1: Effects of different types of substrates on β-1,3 glucanase enzyme activity. Values with the same letter are not significantly different ($P<0.05$). Error bars represent standard deviation from three replicates.

3.2 Production profile of β-1,3 glucanase

The production of extracellular β-1,3 glucanase enzyme was observed during the growth of *P. oxalicum* T3.3 on basal medium containing seaweed as substrate for 7 days (Fig. 2). The highest production of β-1,3 glucanase was obtained at five days of fermentation with activity of 61.13 U mL^{-1}. After the fifth day, there was no further increase in the production of this enzyme. Protein concentration also showed the highest value on the fifth day and decreased thereafter which reflect the presence of enzyme. Fig. 2 also shows the N-acetylglucosamine content of the fungal biomass during 7-days of fermentation by *P. oxalicum* T3.3. The highest glucosamine production was detected on day 3 of fermentation with 2.78 mg g^{-1}. After day 3 of fermentation, glucosamine production continued to decrease. β-1,3 glucanase production was recorded to be maximal after five days of growth. El Katatny *et al*., (2000) also have reported that the production of β-1,3 glucanase enzyme by *Trichoderma harzianum* using laminarin as carbon source was maximum at the fourth day. In another study, *P. oxalicum* showed highest β-1,3 glucanase production on day five (Doughari, 2011). Meanwhile, the results of fungal cell growth was found to be maximal on day three and decreased thereafter as it is may due to the autolysis of the *P. oxalicum*. The results of the study are in a good agreement with Copa-Patino *et al*., (1990) who reported the production of high β-1,3 glucanase activity during autolysis of *P. oxalicum* which increases with incubation time. This activity forms part of a lytic complex

produced by this fungus. Hence, it could be inferred that the production of β-1,3 glucanase is a non-growth associated product.

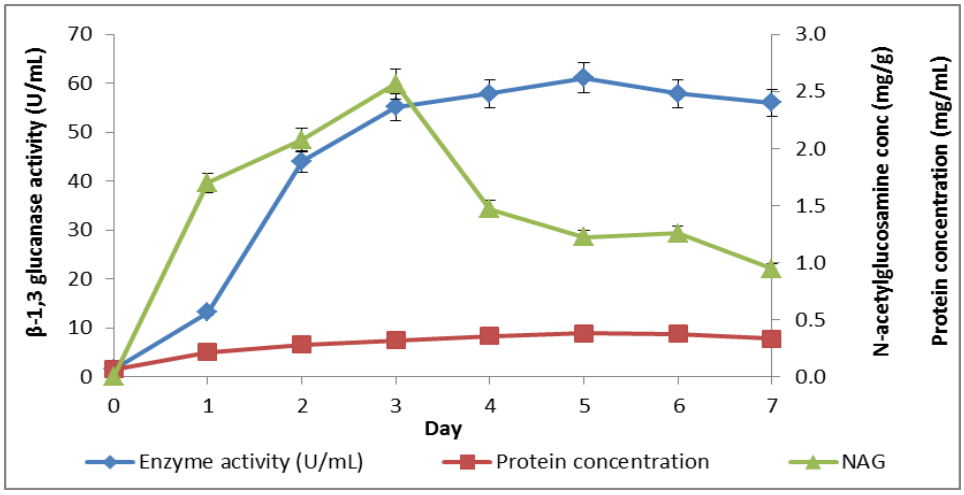

FIG. 2 Production profile of β-1,3 glucanase by *P. oxalicum* T3.3 using seaweed as substrate. Error bars represent standard deviations from three replicates.

3.3 Effects of concentration of substrates

To study the effects of concentrations of substrates, concentrations of seaweed were varied from 0.5% to 3.0%. It was observed that *P. oxalicum* T3.3 produced the highest β-1,3 glucanase in medium supplied with 1% concentration of seaweed (Fig. 3). When the concentration of the seaweed increased above 1%, the production of β-1,3 glucanase decreased. The best β-1,3 glucanase production (70.92 U mL^{-1}) was reached at 1% (w/v) seaweed concentration in the medium. It was found that when the concentration of the seaweed increased above 1%, the production of β-1,3 glucanase decreased as high concentration of substrate might inhibit the production of this enzyme. β-1,3-glucanase production was reported to be significantly affected by substrate concentration which showed that enzyme production increased up to 0.75% of laminarin concentration but decreased at higher concentrations (El Katatny *et al*., 2000). The activity of the enzyme may increase in limited carbon conditions and immediately results in autolysis, as reported in *Botrytis* spp., (Stahmann *et al*., 1992), *P. oxalicum* (Copa-Patino *et al*., 1989) and *Aspergillus nidulans* (Nuero *et al*., 1993). It also seems probable that in conditions of carbon starvation and poor growth this fungus actively secretes some level of hydrolytic enzymes.

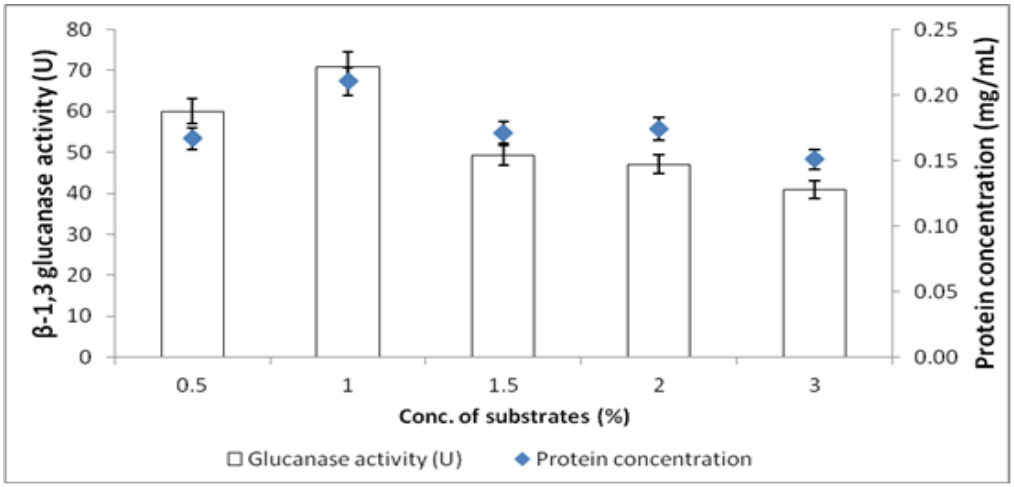

FIG. 3 Effects of concentrations of substrate on production of β-1,3 glucanase. Values with the same letter are not significantly different (P<0.05). Error bars represent standard deviations from three replicates.

3.4 Effects of nitrogen sources

The effect of different nitrogen sources on the production of β-1,3 glucanase enzyme was tested with the medium supplemented with various combinations of peptone, yeast extract and $(NH_4)_2SO_4$. This study was conducted to reduce the number of nitrogen sources used in comparison to the nitrogen sources contained in the basal medium. The combination

consisted of peptone, yeast extract and ammonium sulfate in the ratio of 3:2:2 while control medium contained nitrogen sources in the basal medium before modifications which was 3:0.5:2 respectively. Out of the several nitrogen sources tested, β-1,3 glucanase production was significantly ($p< 0.05$) higher in the presence of a combination of peptone and yeast extract as the nitrogen source (75.55 UmL^{-1}) as compared to the control. Meanwhile in the presence of peptone in the fermentation medium, it contributed to high β-1,3 glucanase production in all samples thus highlighting the importance of peptone in the production of β-1,3 glucanase . On the other hand, the use of ammonium sulfate as the sole nitrogen sources inhibited the production of β-1,3 glucanase. It has been shown that the production of β-1,3 glucanases was also significantly influenced by the nitrogen sources incorporated into the medium (Fig. 4). Combination of peptone and yeast extract was chosen as the most favourable substrate for the production of β-1,3 glucanase because it could reduce the number of nitrogen sources used in the basal medium. Furthermore, this will help to reduce the production cost. From this study, enzyme production was high in medium supplemented with peptone as nitrogen source which was in agreement with the result which reported that peptone-casein was the best nitrogen source for β-1,3 glucanase production, followed by corn steep solid and then NH_4NO_3 (El Katatny, 2000). Moreover, organic nitrogen like yeast extract and peptone significantly increased enzyme production in a novel isolate of *Alcaligenes xylosoxydans* (Vaidya *et al.*, 2001). This result is due to peptone and yeast extract contains abundant of vitamins, minerals, and amino acids, which are necessary for cell growth and enzyme production. On the other hand, the use of ammonium sulfate inhibited β-1,3 glucanase production due to ammonia reducing the culture pH excessively during NH4+ adsorption (Bazilah, 2011). The reduction in culture medium pH was not suitable for fungal growth thus inhibiting the β-1,3 glucanase production. Inhibitory effects of ammonium salts on inulinase production also had been reported by Singh and Gill (2006).

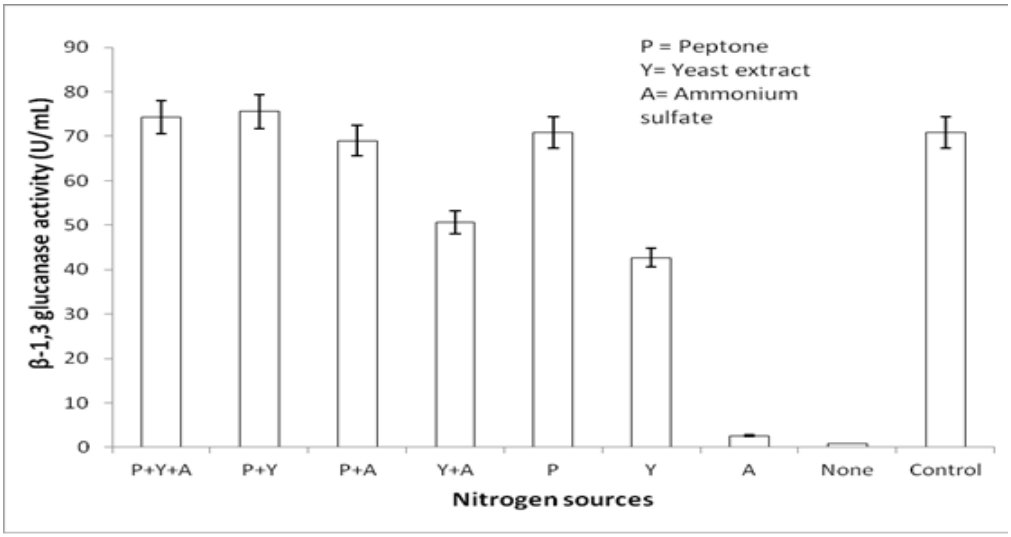

FIG. 4 Effects of nitrogen sources on production of β-1,3 glucanase. Values with the same letter are not significantly different (P<0.05). Error bars represent standard deviations from three replicates.

3.5 Effect of initial pH medium

Based on Fig.5, the medium with initial pH set range between 4.5 to 5.5 showed high β-1,3 glucanase production with the highest β-1,3 glucanase production measured when the initial pH of the medium was set to pH 5.0 (79.04 UmL^{-1}). Meanwhile, the maximum protein concentration was achieved at pH 5.5. Lower β-1,3 glucanase production was detected when the initial medium pH was set to pH 4.0 and 6.0. At pH 4.0 and 6.0, cell concentration was significantly ($p< 0.05$) reduced. Hence, it was concluded that the production of β-1,3 glucanase was favourable at initial pH range between pH 4.5 to 5.5. Culture pH is one of the factors to affect enzyme production in other fungi. From this study, β-1,3 glucanase production was favored by acidic pH range between pH 4.5 and 5.5. However, initial medium pH below 4.5 and above 6 may inhibited the production of β-1,3 glucanase. This is due to the majority of fungi not being able to grow in strongly acidic or strongly alkaline conditions accounting for the loss of enzyme activity. It was also reported that acidic pH is one of the essential growth factors in the production of β-1,3 glucanases and chitinases in thermophilic *Streptomyces* and in the mycoparasite *T. harzianum* respectively (Tweeddel *et al.*, 1993). Initial medium pH affected production of β-1,3 glucanase in *T. harzianum* with optimum β-1,3 glucanse production on laminarin being highest when the initial pH was set to pH 5.5 (El Katatny *et al.*, 2000).

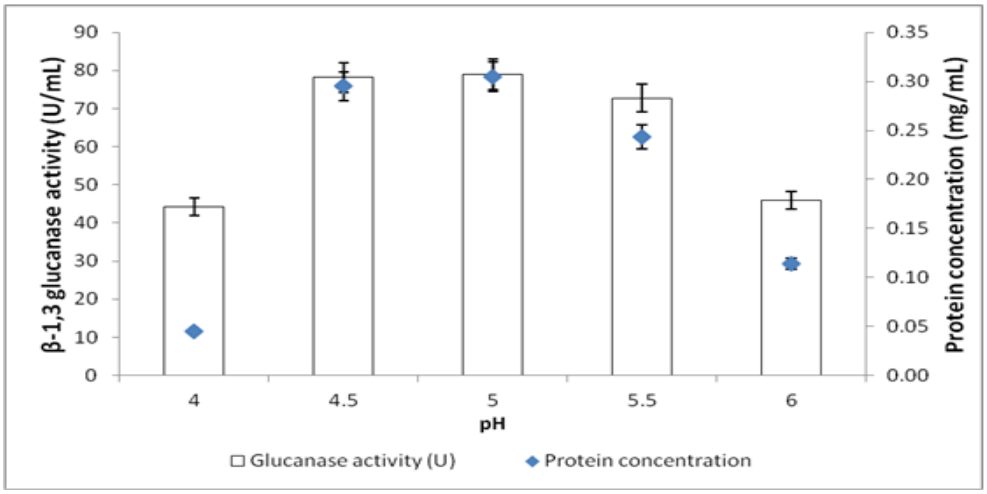

FIG. 5 Effects of initial medium pH value on production of β-1,3 glucanase. Values with the same letter are not significantly different (P<0.05). Error bars represent standard deviations from three replicates.

3.6 Effect of agitation speed

β-1,3-glucanase production was also significantly influenced by the agitation speed and the highest β-1,3-glucanase production was obtained at 200 rpm (Fig. 6). A significant ($p< 0.05$) increase in β-1,3 glucanase activity (80.03 UmL^{-1}) was observed at agitation speed of 200 rpm compared to stationary condition which was 0 rpm (0.965 UmL^{-1}). The protein concentration also showed maximum value at 200 rpm. At 0 rpm, almost no β-1,3 glucanase was produced and the protein concentration was also at its lowest. It was concluded that agitation exerted a strong influence on production of β-1,3 glucanase. The effect of the agitation speed on the extracellular protein concentration (Fig. 6) was also the same as on the enzyme activity. In this study, seaweed substrate did not dissolve in the production medium, so there was interaction between substrate and fungus in the medium. However, it was observed that at 0 rpm, this fungus only grew at the surface of the medium and had no interaction with the substrate. Thus, at 0 rpm almost no β-1,3 glucanase was produced and the protein concentration was also shown to the lowest because the uptake of the substrate was very low due to poor mixing and agitation. High agitation rate is important in fungal fermentation to provide adequate mixing and mass transfer (Abd-Aziz *et al*., 2008). On the other hand, at 120 rpm β-1,3 glucanase activity was much more lower compared to at 200 rpm. This resulted as a consequence of inadequate mixing and/or resistance in mass transfer at lower agitation speed (Arthur and Panda, 2000). It was reported by Lejeune and Barone (1994) that the optimal agitation rate for enzyme production of *T. reesei* was 200 rpm, whilst 300 rpm was recorded as showing the fastest growth. β-1,3 glucanase is the most affected and sensitive to agitation speed, followed by CMCase, and xylanase being the least affected.

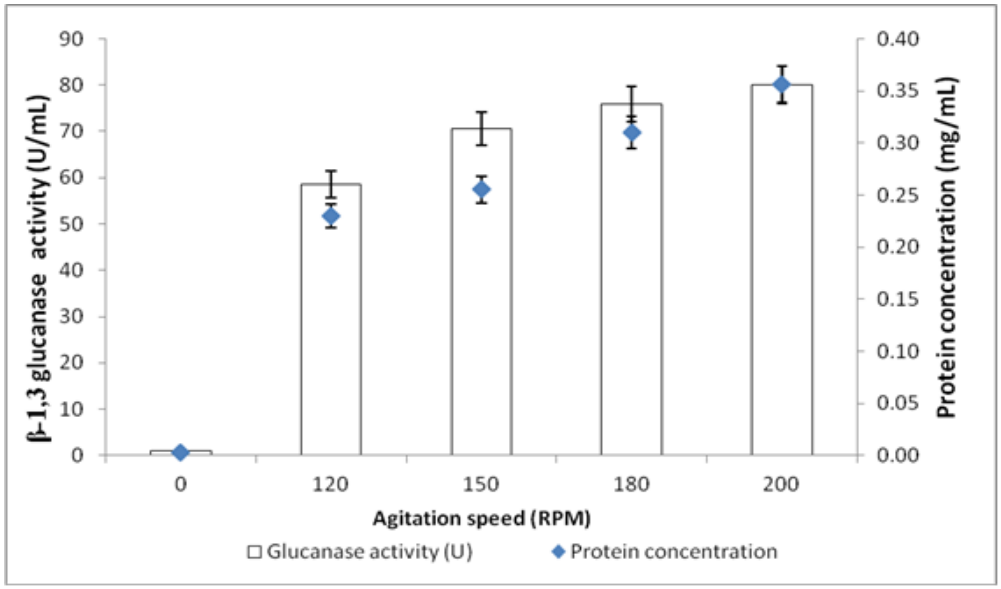

FIG. 6 Effects of agitation speeds on production of β-1,3 glucanase. Values with the same letter are not significantly different (P<0.05). Error bars represent standard deviations from three replicates.

3.7 Effect of surfactants

Two different groups of surfactants; anionic such as sodium dodecyl sulphate and non-ionic such as Triton X-100, Tween20 and Tween80 were tested for β-1,3 glucanase production. From this study it has showed that sodium dodecyl sulfate, Tween 80, Tween 20 were found to increase β-1,3 glucanase production with sodium dodecyl sulfate showing the best result (84.73 UmL^{-1}) (Fig. 7). β-1,3 glucanase production was significantly ($p< 0.05$) higher in medium supplemented with sodium dodecyl sulfate as compared to control which did not contain any surfactant. Triton X-100 was found to decrease β-1,3 glucanase production. These results showed that surfactants also gave significant effects on the production of β-1,3 glucanase. β-1,3 glucanase production was enhanced significantly when surfactants were added into the production medium. In general, surfactants may increase the cell membrane permeability through decreasing their phospholipid contents and solubilization of membrane bound proteins. Among the different surfactants, sodium dodecyl sulfate showed best results. This result is in agreement with the study by Singh and Bhermi (2008), which reported that sodium dodecyl sulfate (SDS) has an enhancing effect on enzyme production However, Triton X-100 showed decrease in β-1,3 glucanase production. This finding is in agreement with the results obtained by Pardo, (1996) on cellulase production by *Nectria catalinensis*. It was found that, triton X-100 had an enhancing effect on cellobiose activity, but is inhibited endoglucanase and exoglucanase production. Tween 80 could help in increasing cell membrane permeability leading to a more efficient uptake of nutrients, without any significant change in oxygen supply. Surfactants also had been found to facilitate the entry of compounds into cells. It was also observed that surfactants changed the cell membrane structure to assist enzyme release (Liu *et al*., 2006). Through this optimization process, it were shown that under the optimized conditions, β-1,3 glucanase activity increased 38.6%.

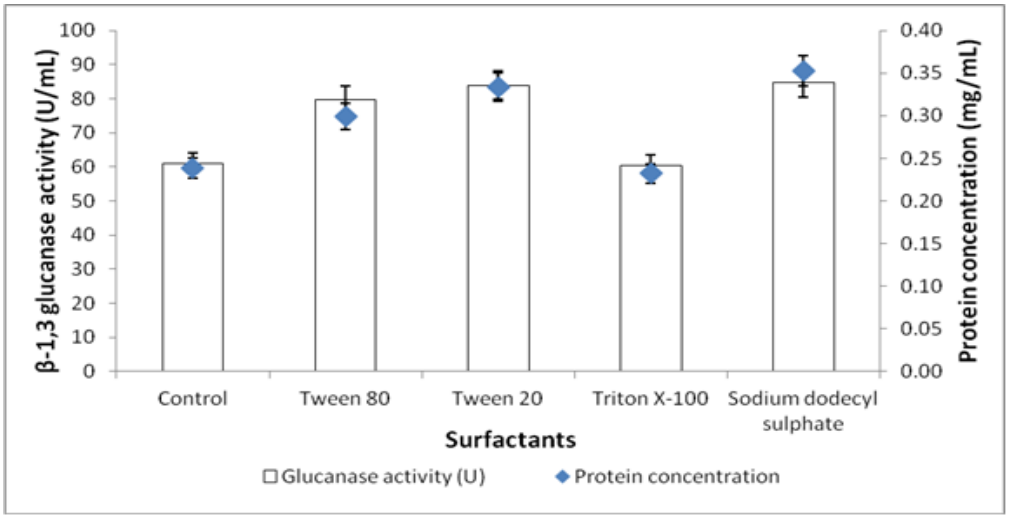

FIG. 7 Effects of surfactants on production of β-1,3 glucanase. Values with the same letter are not significantly different (P<0.05). Error bars represent standard deviations from three replicates.

IV. CONCLUSION

The optimization of β-1,3 glucanase production by *P.oxalicum* required adjustment of different types of carbon and nitrogen sources, initial pH medium, agitation speed and surfactants. This enzyme characterization has revealed its great potential towards detergent, beer and food fermentation industries whose manufacturing conditions are largely acidic.

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Synergistic Effects of Diazotrophic Phosphate Solubilizing *Azotobacter chroococcum* and AM Fungus *Glomus mosseae* on Yield Improvement in Finger Millet (*Eleusine Coracana* (L.) Gaertn.)

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Abstract*—Field experiments were conducted to study the response of finger millet Eleusine Coracana* (L.) Gaertn. *to combined inoculation with the P-solubilizing diazotrophic Azotobacter chroococcum (DT) isolated from the rhizosphere of finger millet and the P-mobilizing Arbuscular Mycorrhizal fungus Glomus mosseae under graded levels of N & P fertilizers (100, 75 and 50 percent).The inoculated seedlings were transplanted to the laid out plots (RCBD) with red sandy loam soil having medium levels of NPK and OC. The combined inoculation resulted in significant increase in yield over all the other treatments with 31.00, 27.99 and 18.10 percent grain yield and 36.69, 33.55 and 15.01 percent straw yield respectively in presence of 100, 75 and 50 percent N and P fertilizers in comparison with uninoculated control indicating efficient N supplementation and P- solubilization by A. chroococcum and P-mobilization by the AM fungus. The results indicated that 25-50 percent of N and P fertilizers can be curtailed in presence of combined inoculants, saving money without decrease in the yield performance of finger millet. The combination of these inoculants can be included as one of the important agronomic inputs in finger millet cultivation both under subsistence and affluent farming systems*.

Keywords—Azatobacter chroococcum, Biofertilizers, Eleusine coracana, finger millet, Glomus mosseae.

I. INTRODUCTION

Finger millet (*Eleusine Coracana* (L.) Gaertn.) popularly known as ragi is one of the important minor millets grown in India and ranks second in importance among the millets cultivated in India assuming growing importance due to its dietary role among rural folk and the diabetics. The crop is grown in 2.5 million hectares in the country and Karnataka ranks first with an area of 0.99 m. ha under ragi cultivation [1]. To a large extent, the finger millet is cultivated by poor and marginal farmers under subsistence farming with low nutrient input. Nitrogen and phosphorus determine the yield in combination with potash. However, the soils grown for ragi are of low fertility soils resulting in wide regional variations in yield averaging 1500-1700 kg/ha as against the varietal potential of 4000 to 5000kgs. Nitrogen fixing Bacteria (NFB), phosphate solubilizing bacteria (PSB) and Arbuscular Mycorrhizal Fungal (AMF) inoculants play a major role in supplementing and mobilizing these nutrients to plants under low input conditions while reducing their application under affluent farming systems. To harness the synergistic effects, these three groups of inoculants have to be used in combination which is not a common practice among farmers. However, this problem can be solved to a great extent by using bacterial isolates which perform both the functions of nitrogen fixation and phosphate solubilization. In nature there exist bacteria which perform dual functions of nitrogen fixation and phosphate solubilization in a single application. Such phosphate solubilizing diazotrophs among *Azotobacter, Bacillus* and *Azospirillum* species have been reported from the rhizosphere of plants Halder *et al.,* [2], Abd alla [3][4], Sheshadri *et al.,* [5]; Vivek Kumar *et al*.,[6]; Monica *et al*.,[7]; Rahim *et al.,* [8] and Hafsa *et al*.,[9]. Chandana and Venkataramana [10] isolated and evaluated phosphate solubilizing diazotrophic *Azotobacter chroococcum* and *Bacillus* sp. from the rhizosphere of finger millet with significant grain and straw yield improving influences. A few reports are available on the effects of tripartite inoculation of NFB, PSB and AMFs with encouraging results [11]. However there is lack of information on the synergistic influences of phosphate solubilizing diazotrophic *Azotobacter chroococcum* in combination with AM fungus *Glomus mosseae* on finger millet. The present study reports for the time the growth and yield response of finger millet to the combined inoculation with phosphate solubilizing diazotrophic *Azotobacter chroococcum* (DT) in combination with AM fungus *Glomus mosseae.*

II. MATERIAL AND METHOD

The field experiments were conducted at the campus of M/s. Chaitra Biofertilizers and Chemicals (P) Ltd., Mysore, Karnataka, India during 2016-2018 using the finger millet variety MR1 under irrigated condition. The experiment was laid out randomized complete block design with three replicates per treatment. Each plot was 2 x 1 m size. The recommended fertilizers are NPK 100:50:50 kg/ha. There were 12 treatments and all the treatments received 10 tons of farmyard manure and full dose of potash fertilizer (50 kg/ha) while N & P fertilizer dosage varied (100, 75, 50 percent/ha) depending on the treatments. *A. chroococcum* (DT) having the dual trait of nitrogen fixing and phosphate solubilization, isolated from the rhizosphere of finger millet and evaluated under pot culture conditions (unpublished) was used in comparison with uninoculated control. The lignite based inoculant of *A. chroococcum* (DT) was used at 25gm/kg seeds with carboxy methyl cellulose as sticker. The treatments imposed are given in the table 1.

The treated seeds were sown in nursery trays with cavities and irrigated on daily before transplantation to the field. Prior to sowing, the potting material (sterilized cocopith) was inoculated with one gram of *G. mosseae* inoculum containing 20 spores/gm of carrier material depending on the treatment imposed. Care was taken to avoid cross contamination. The 20 days old seedlings were transplanted along with the soil holding the seedlings in the nursery tray cavities, to the holes made in the soil at 22.86 x 15.24cms spacing in each plot. The plots were irrigated once in 8-10 days depending on the rains received. Data on plant height and number of productive tillers were collected on 90^{th} day selecting 8 plants/replicate avoiding the boarder rows. Harvest was made on 120^{th} day from each replicate on whole plot basis avoiding the boarder rows. Grain weight/earhead, grain and straw yield were collected after sun drying till a constant weight was obtained. Yield per hectare was calculated. The data was statistically analyzed following the method developed by Sundaraj *et al.*,[12].

III. RESULTS AND DISCUSSION

The results obtained on the response of finger millet to combined inoculation with the phosphate solubilizing diazotrophic *Azotobacter chroococcum* (DT) and phosphate mobilizing AM fungus *Glomus mosseae* are presented in the table 1. Significant differences were recorded on the following parameters studied.

TABLE 1
RESPONSE OF FINGER MILLET TO DUAL INOCULATION WITH P SOLUBILIZING DIAZOTROPHIC *AZOTOBACTER CHROOCOCCUM* (DT) AND AM FUNGUS *GLOMUS MOSSEAE* UNDER FIELD CONDITIONS (AVERAGE DATA OF FOUR HARVESTS)

Treatments	Plant height (cm)	No. of Productive tillers	Grain weight/ Earhead (gm)	Grain yield/ha (kg)	Straw yield/ha (kg)
T1 Uninoculated control - 100% N & P	112	4.25	4.53	3890	8947
T2 75% N & P	99	4.17	3.84	3210	7704
T3 50% N & P	70	4.13	2.88	2380	5712
T4 100% N & P + *Ac* (DT)	121	4.30	5.15	4429	10629
T5 75% N & P + *Ac* (DT)	108	4.28	5.00	4280	9416
T6 50% N & P + *Ac* (DT)	106	4.19	4.93	4128	8668
T7 100% N & P + *Gm*	118	4.30	4.66	4010	9223
T8 75% N & P + *Gm*	100	4.26	4.27	3651	8560
T9 50% N & P + *Gm*	80	4.25	3.35	2888	6931
T10 100% N & P + *Gm* + *Ac*	130	4.31	5.85	5096	12230
T11 75% N & P +*Gm*+ *Ac*	124	4.27	5.75	4979	11949
T12 50% N & P + *Gm*+ *Ac*	120	4.28	5.30	4594	10290
CD @ 5%	**4.20**	**0.05**	**0.60**	**453.02**	**573.00**

Ac- Azotobacter chroococcum; Gm- Glomus mosseae

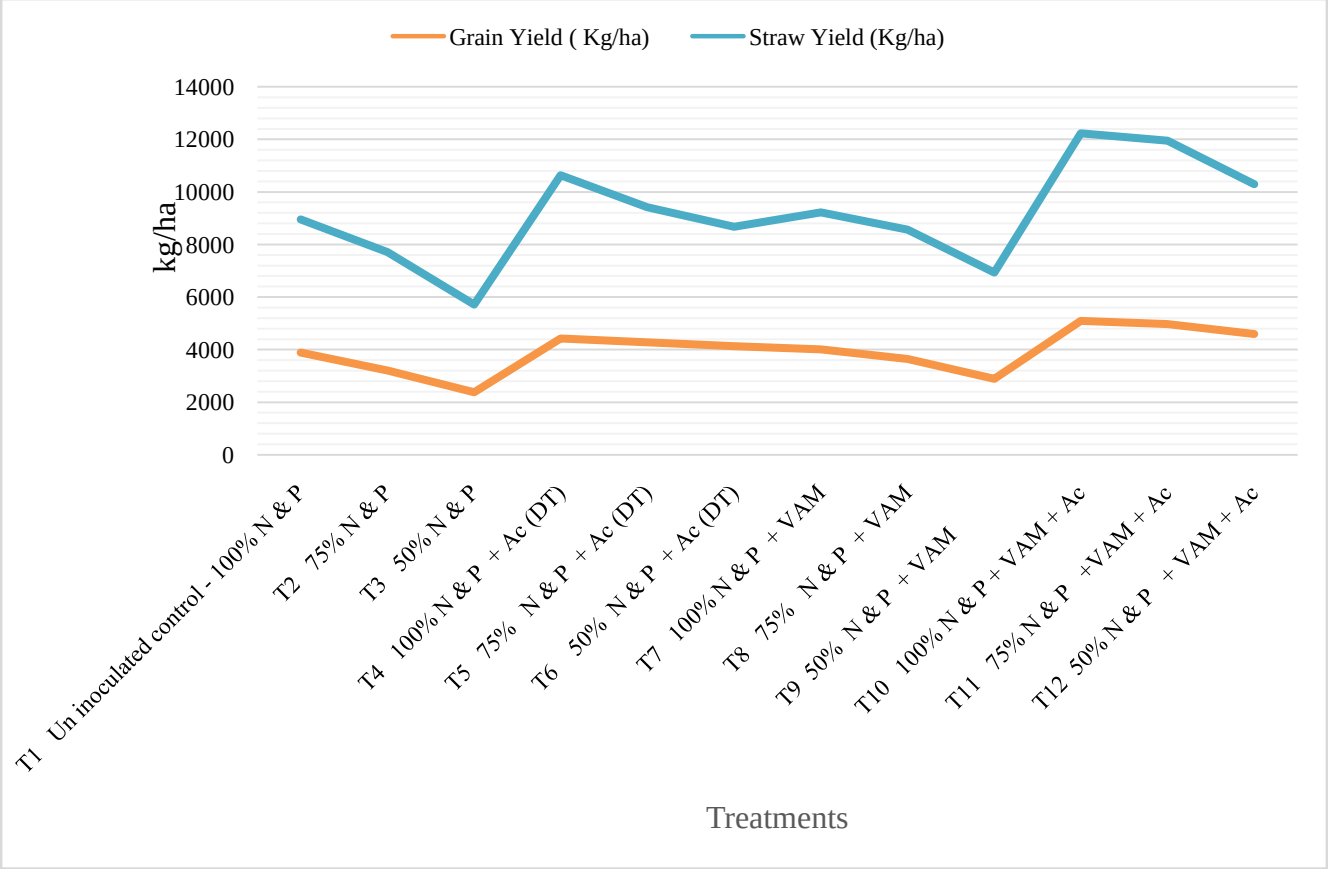

FIGURE 1: Grain and Straw yield in finger millet in response to combined inoculation with *A.chroococcum* (DT) and *G. mosseae*

3.1 Growth Parameters

Plant height varied significantly in response to combined inoculation in treatments T-10 (130 cm), T-11 (124 cm) and T-12 (120 cm) provided with 100, 75 and 50 percent of the recommended dose of N & P fertilizers (100 : 50 kg/ha) as compared to the uninoculated control T-1 (112 cm) receiving 100% recommended N and P fertilizers. The increase recorded was 16.07, 10.71 and 7.14 percent respectively over control. The plants receiving individual inoculants of *A. chroococcum* (DT) and *G. mosseae* (T-4 and T-7) in presence of 100% N and P also recorded significant increase in height (121 and 118 cm) over control but not over T-10 but were on par with T11 receiving combined inoculation in presence of 75% N and P. Plant height was not significant over control in T-5 and T-8 receiving 75% N and P and T-6 and T-9 with 50% N and P in all treatments inoculated singly with *A. chroococcum* (DT) or *G. mosseae.*

The number of productive tillers per plant ranged from 4.13 in uninoculated treatment (T-3) receiving 50% N and P to 4.31 in T-10 receiving combined inoculation and 100% N and P. The plants receiving combined inoculation in presence of 100% N and P (T-10) produced significantly higher number of tillers (4.31) compared to control and other treatments. The number of tillers in plants provided with single inoculants and 100% N & P were on par with control. Other treatment was not significant with control.

3.2 Yield parameters

The grain weight per earhead, grain and straw yield per hectare recorded more significant differences among treatments. Grain weight per earhead in plants inoculated combined in presence of graded levels of N and P fertilizers (T10, T11 and T12) increased significantly (5.85, 5.75 and 5.30gm) over control T1 (4.53 gm) by 29.14, 26.93 and 17.20 percent respectively. Among the treatments receiving individual inoculations with 100% N & P, only T4 inoculated with *A. chroococcum* (DT) recorded significant increase (5.15gm) over control with an increase of 13.68% while other treatments were less significant compared to control.

Similar to the trend observed in grain yield per earhead, the grain yield per hectare also showed significant differences among different treatments. Highest increase was recorded in T10 inoculated combined in presence of 100% N and P fertilizers

yielding 5096 kg/ha followed by T11 (4979 kg) and T12 (4594 kg) provided with 75 and 50 percent N and P inoculated together with *A. chroococcum (DT)* and *G. mosseae* registering an increase of 31.00, 27.99 and 18.10 percent respectively over control. Among the singly inoculated treatments in presence of 100% N and P, T4 with *A. chroococcum* (DT) yielded 4429 kg/ha which was significant over control with an increase of 13.85% while the same inoculant with 75 and 50 percent N and P (T5 and T6) recorded yield which was on par with control but with an increase of 10.02 and 6.2 percent respectively. Other individually inoculated treatments were not significant over control.

Straw yield also registered an improvement in treatments corresponding to those treatments showing increased grain yield. Significant increase was recorded in three treatments T10, T11 and T12 receiving combined inoculation in presence of all the three levels of fertilizers. The yield was 12230, 11949 and 10290 kg/ha, an improvement of 36.69, 33.55 and 15.01 percent over control. The yield levels were significant over all the other inoculated and uninoculated treatments. Straw yield in response to single inoculation with *A. chroococcum* (DT) in presence of 100% N and P (T4) was significant over control with 10,629 kg/ha, an increase of 18.80% while with 75 and 50% N and P (T5 and T6) the yield was on par with control recording 9416 and 8668 kg/ha an increase of 5.24 and 3.12 percent respectively. Straw yield with *G. mosseae* as single inoculant in presence of 100% N and P was on par with control (T1) with 9223 kg/ha, an increase of 7.57% while treatments T8 and T9 with 75 and 50% N and P were not significant over control (fig 1).

The present study reports the synergistic interactions of phosphate solubilizing diazotrophic *A. chroococcum* and the P mobilizing AM fungus *G. mosseae* on grain and straw yield improvement in finger millet. The synergistic interactions between plant growth promoting bacteria and AM fungi on crop plants are on account of a wide range of mechanisms including nitrogen supplementation and available P transportation especially in low fertility soils. A few reports are available on possession of dual traits of P solubilizing abilities in *Azotobacter*, *Azospirillum* species among nitrogen fixers and nitrogen fixing abilities in *Bacillus* species among phosphate solubilizing bacteria and their positive influence in improving crop yield in wheat, maize and finger millet (Kumar *et al*., [13]; Monica *et al*., [7]; Rahim *et al*.,[8]; Chandana and Venkataramana, [10]). Synergistic interactions between AM fungi and *Azotobacter* having single trait of nitrogen fixation have been reported by Bhagyaraj [11]; Kumar *et al*., [13] ; Rishi *et al*., [14]. However, there are no such studies on the synergistic interactions involving P-solubilizing diazotrophic *A. chroococcum* in association with AM fungi in crop plants in general and finger millet in particular. This is the first report on such interactions between P solubilizing, *A. chroococcum* and AM fungus *G. mosseae* on finger millet. Several possible mechanisms mediating the interactions have been reported such as nitrogen fixation and hormone production by *Azotobacter* and phosphate acquisition, uptake and transportation by AM fungi and both together stimulating root hair and lateral root formation, and increased root biomass production, expanding the nutrient and water absorptive capacity of root system in the soil and also increased root colonization by AM fungus (Bhagyaraj [11]; Rishi *et al*., [14]). The results obtained in the present study suggest such interactions between *A. chroococcum* (DT) and *G. mosseae* reflecting on the increased grain and straw yield in finger millet. Phosphorus is critical for biological nitrogen fixation (Singleton *et al*., [15] and Arun[16]).The presence of both the traits in *A. chroococcum* (DT) act complementarily in increasing nitrogen fixation as the phosphorus required is made available by the same bacterium through P-solubilization. The AM fungi not only absorb the soil available P but also the P released through solubilization process by the bacterium. The increments in grain and straw yield recorded in the present study can be attributed to the above mechanisms. The study also reports an important finding that 25 to 50% of N and P fertilizers can be reduced in presence of these organisms thus saving money on fertilizers without reduction in the yield parameters. It can be concluded that the P solubilizing diazotrophic *A. chroococcum* (DT) can be the next generation microbial inoculant for sustainable finger millet production with enhanced activity in combination with AM fungus *G. mosseae*. The combination not only helps in supplementing the N & P nutrients under subsistence farming but also to reduce their application under affluent farming.

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