STUDY ON EFFECT OF CHITOSAN OLIGOSACCHARIDE FERTILIZER IN CULTIVATION OF *Piper betle* L.(BETEL VINE)

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ABSTRACT

The study was carried out to provide the effect of different concentrations of Chitosan Oligosaccharide fertilizer on growth and yield percent in leaf of *Piper betle*Linn belongs to family *Piperaceae* commonly known as betel vine. The plant is locally known as Kun in Myanmar. The effects of different concentration of chitosan oligosaccharide fertilizer on cultivation of *P.betle*plant were studied. The phytochemical screening and antimicrobial activity of *P. betle*leaf were also studied. The yield percent of leaf *P. betle*showed significantly increased in chitosan fertilizer used plants. Phytochemical investigation of *P. betle* leaf revealed the presence of alkaloids, glycoside, carbohydrate, amino acid, phenolic compound, flavonoid, steroid, terpenoid, saponin, tannin, starch, reducing sugar and organic acid. From the results of antimicrobial activity assay, it was observed that ethyl acetate and ethanol extract of chitosan oligosaccharide fertilizer used of *P. betle*leaf have most powerful antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacilliuspumilus, Candida albicans andE-coli*.

Key words: *Piper betle*, Chitosan Oligosaccharide, yield percent, phytochemical, antimicrobial

INTRODUCTION

Betel (*Piper betle*L.) is locally known as Kunand Betel in English. It belongs to the genus *Piper* of the *Piperaceae* family. Its heart-shaped leaves can grow up to the size of 18 cm in length and 12 cm in width. This plant is cultivated most parts of South India, Bengal, Sri Lanka, Myanmar and Thailand for its leaves. Since it is a creeper, it needs a compatible tree or a long pole for support. Fertile soil is best for *P. betle*cultivation. The farm yard is fenced with bamboo sticks and coconut eaves. The creeper cuttings are planted after proper dressing. The plants are neatly arranged in parallel rows about two feet apart and the saplings are twined around up right sticks of split bamboo and reeds. Proper shade and irrigation are essential for the successful cultivation of this crop. The plants are regularly watered in the hot months. Generally dry cow dungs were applied as organic fertilizer. Recently farmers were using chemical fertilizer (NPK) as mixed fertilizer. Though chemical fertilizer application increases betel leaves production, but in dry season lock of mulching and watering the betel leaves vines become dead. Most of farmers avoid use of chemical fertilizer fearing degradation of soil nutrient. In fact, the advancement of technological for cultivation and better understanding of the habit and habitual of plants are even greater increase in time to come (Nikhi Kumar, 1999).

Chitosan Oligosaccharide water soluble powder (agriculture grade) is a mixture of oligomers of D-glucosamine. It is only known positively charged alkaline amino oligosaccharide which is derived from rich marine biological resources(shrimp and crab shells) with enzymatic degradation. Characteristics of Chitosan Oligosaccharide fertilizer are nature safe and biological decomposable, effective and reliable for crops, vegetables, fruits and flowers, active plants protection against fungal, bacterial, virus diseases, promote the plant growth,improve yield (mineral absorption accelerant)and improve the soil structure (Yin *et al.,* 2016)

Therefore, effectof Chitosan Oligosaccharide saccharide fertilizer on cultivation of *P.betle* plant were studied.

Medicinal Properties of *P. betle* L.

Betel leaves are the most important plant part and it possesses medicinal, religious and ceremonial value in Southeast Asia. In India, it is customary to serve betel leaf on various social, cultural and religious occasions and is also offered to guests as a mark of respect (Warrier*et al.,* 1995). Fresh betel leaf is chewed together with areca nut and slaked lime as natural tonic and breath refresher. Aqueous extracts of *P. betle*have also been shown to reduce the adherence of early dental plaque bacteria (Razak *et al*., 2006). As well as use as a mouth freshener, the leaves are used for wound healing and digestive and pancreatic lipase stimulant activities in traditional medicine (Prabhu *et al*., 1995). Antioxidant, anti-bacterial and anti-fungal, antiinflammatory,anti-diabetic and radioprotective activities of *P. betle*have also been reported(Chakraborty and Shah, 2011).

MATERIALS AND METHODS

Cultivation of *P. betle* Plants

In the present research, the *P.betle*plant (Kun) was cultivated closed system cultivation using rectangular structure by applying Chitosan Oligosaccharide fertilizer(Qingdao Shellight Biotechnology CoLtd., China) in Kungyangone Township, Yangon Region. *P.betle*plants were propagated through stem cutting. The plants were then placed in tidily arranged rows at about a feet distance in parallel rows. After 15 days, each row of plants wastreated by different concentration (0 ppm, 25 ppm, 50 ppm, 75 ppm) of Chitosan Oligosaccharide fertilizer. The study was conducted during 2017 June to 2018 January. Yield percent of leaf was studied at this cultivation period.

Determination of Elemental Compositions by EDXRF

Some trace elements of *P.betle*leaves powderwere determined by using Energy Dispersive X-Ray Fluorescence (EDXRF). The leave samples were analyzed directly in their powder solid form by Shimadzu Energy Dispersive X- Ray Fluorescence Spectrometer (EDX-8000). The determination was performed at Department of Chemistry, West Yangon University, Myanmar.

Preliminary Phytochemical Investigation of *P. betle*

Phytochemical investigations were performed to know the different types of chemical constituents such as alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, cyanogenic glycosides, starch, reducing sugars, organic acids and quinones according to the appropriate reported procedure (Harbone, 1984).

Screening of Antimicrobial Activity of Various Extracts from *P. betle*

The antimicrobial activities of different crude extracts of*P.betle*such as petroleum ether, ethyl acetate, ethanol and water extracts were determined against six microorganisms such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*and *Escherichia coli* species by employing agar well diffusion method at Pharmaceutical Research Department, Ministry of Industry , Yangon, Myanmar (Mar Mar Nyein *et al*, 1991).

RESULTS AND DISCUSSION

Effect of Chitosan Fertilizer on *P. betle* Growing

The betel leaf is usually cultivated following the traditional methods. It is constructed with the locally available materials like bamboo stems, jute sticks, paddy straw, petioles and leaves of banana etc. In the present research, *P.betle*(kun) were planted by the treated of Chitosan Oligosaccharide fertilizer and not treated Chitosan Oligosaccharide fertilizer (control). Each betel plant has 3 betel leaf (Figure 1) at initial cultivation period. Chitosan Oligosaccharide fertilizer of 25 ppm, 50 ppm and 75 ppm solutions were treated after 15 days.The number of betel leaves was found to be gradually increased and grown well in Chitosan treated plants. After 60 days, 25 ppm, 50 ppm and 75 ppm Chitosan Oligosaccharide fertilizer treated plants gave maximum leaves in plant(Figure 2) and they can be also plucked from plant and theremaining leaf become mature andplucked every 15 days after first plucking. Control betel plants were plucked after planting 120 days. Figure 3 markedly showed that growing of betel leaves ofChitosanOligosaccharide 25 ppm, 50 ppm, and 75 ppm treated plant and control plant after 135 days.Yield of leaves were also recorded in Table 1. After 135 days, its yields were found 4.96 kg, 6.02 kg and 5.67 kg and 1.75 kg for Chitosan Oligosaccharide 25 ppm, 50 ppm and 75 ppm treatedbetel plants and control plants (0 ppm), respectively. Therefore, Chitosan Oligosaccharide fertilizer used plants were found to be productivity increased, any insets fall on plant and smooth the leaves.

Figure 1 *P. betle* Plantation at Initial Cultivation Stage

Figure 2 *P.betle* Plantation treated with different concentration of ChitosanOligosaccharide (0ppm, 25 ppm, 50 ppm, 75 ppm) after 60 days cultivation

Figure 3 *P.betle*Plantation treated with different concentration of ChitosanOligosaccharide (0ppm, 25 ppm, 50 ppm, 75 ppm) after 135 days cultivation

Table 1 Yield of *P.betle*Leaf during Cultivation Period

	Average yield (kg)						Total weight (kg)
Day / CS(ppm)	60	75	90	105	120	135	
0(control)	-	-	-	-	0.83	0.92	1.75
25	0.83	0.81	0.84	0.78	0.84	0.86	4.96
50	0.95	0.96	1.02	0.98	0.99	1.12	6.02
75	0.89	0.94	0.96	0.89	0.98	1.01	5.67

Elemental Compositions of *P.betle*

The relative abundance of element in leaves sample of *P.betle*(Figure 4) was carried out by using Shimadzu Energy Dispersive X-Ray Spectrometer (EDX-8000) at Chemistry Department, West Yangon University. The result of betel leaves powder (not treated Chitosan fertilizer and Chitosan treated)are shown in Table 2. Form the results, the organic compounds were predominantly in both samples, 95.811 and 90.934 %, respectively. Among them K content was relatively found that 1.628 % and smaller amount of P, S , Fe, Mn were also found in betel leaves powder of not treated ChitosanOligosaccharide. The contents elements present in ChitosanOligosaccharide treated plantof betel leaves powder, K the content was 5.025 %. Ca, S, P and Cu and Sr were also found as more content than that of not treated ChitosanOligosaccharide. From this result, element contents of betel leaves in treatedof ChitosanOligosaccharide fertilizer plantwere more composed than not used Chitosan Oligosaccharide fertilizer.These elements are essential for maintain of good health.

Phytochemical Investigation of *P.betle*leaf Powder

The preliminary phytochemical investigation of *P.betle*leaves treated of chitosan oligosaccharide fertilizer was carried out to view the different types of chemical constituents present in the leaves according to the procedures. The results are shown in Table 3. From these results, it was observed that alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, starch, reducing sugars and organic acids were present in the leaves of *P.betle.* These phytochemicals are effective for human health.

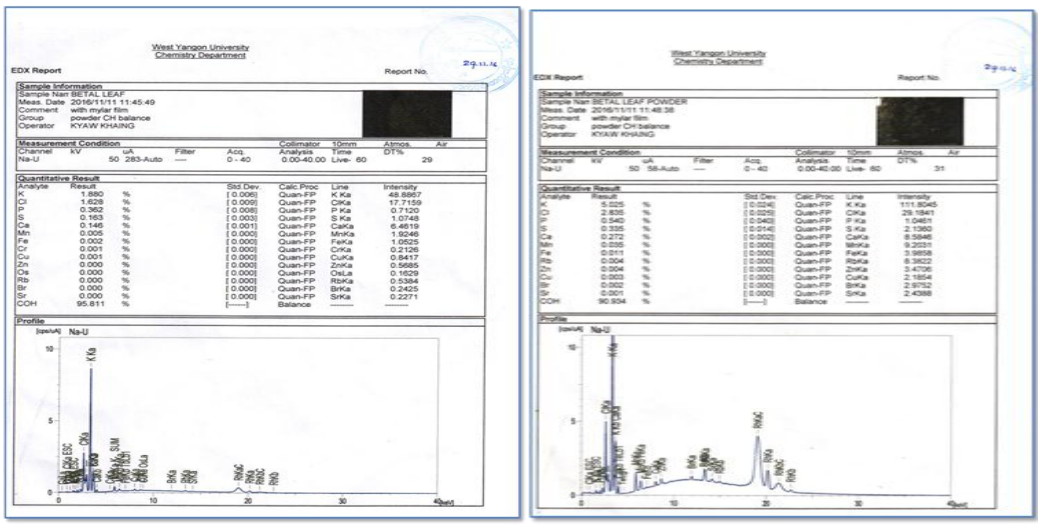


Figure 4 EDXRF spectrum of *P betle* leaf powder (not used and used Chitosan Oligosaccharide fertilizer)

Table 2 Relative Abundance of Mineral Element in *P.betle*Leaf

Element	Relative Abundance (%)	
	(not treated Chitosan Oligosaccharide fertilizer)	(treated Chitosan Oligosaccharide fertilizer)
K	1.880	5.025
Cl	1.628	2.835
P	0.362	0.540
S	0.163	0.335
Ca	0.146	0.272
Mn	0.005	0.035
Fe	0.002	0.011
Rb	-	0.004
Zn	-	0.004
Cu	0.001	0.003
Br	-	0.002
Sr	-	0.001
COH	95.811	90.934

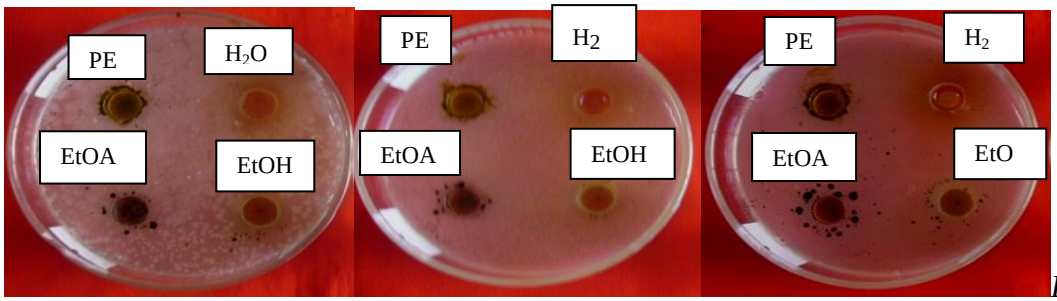
Table 3 Results of Phytochemical Constituents of *P.betle*Leaf

Chemical Constituents	Test Reagent	Observation	Inference
Alkaloids	(i) Dragendorff's	Orange ppt.	+
	(iii) Mayer's	White ppt.	+
α-amino acids	Ninhydrin	Purple colour	+
Carbohydrates	Molisch's	Violet ring	+
Cyonogenic Glycoside	Sodium picrate	No change	-
Flavonoids	Shinoda's	Pink solution	+
Glycosides	10 % lead acetate	Brown ppt.	+
Organic acids	Bromocresol green	Yellow solution	+
Phenolic compound	5 % ferric chloride	Brown ppt.	+
Quinones	Hydrochloric acid	Yellow colour	+
Reducing sugars	Benedict's	Light green ppt.	-
Saponins	Foam test	Marked frothing	+
Starch	Iodine	Red	+
Steriods	LibermanBurchard	Greenish yellow solution	+
Tannins	Ferrous sulphate	Green ppt	+
Terpenoids	LibermanBurchord	Pink	+

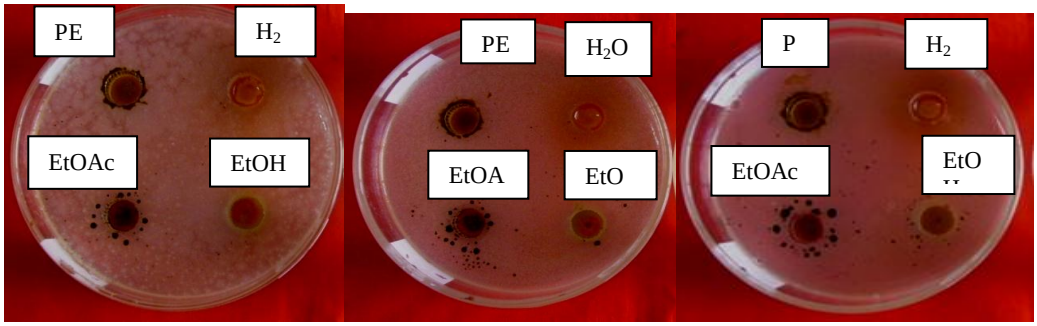
*(+ indicates present, - indicates absent)

Screening of Antimicrobial Activity of Various Crude Extracts from *P. betle*

In vitro screening of antimicrobial activity of various extracts such as petroleum ether, ethyl acetate, ethanol and water extracts from *P. betle*leaf treated of chitosan oligosaccharide fertilizer were done by agar well diffusion method according to the procedure (Figure 5) tested on against six microorganisms such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*and*Escherichia coli.*All extracts of *P. betle*were active on test microorganisms. In the sample PE extract is found low activity on the all organisms and water extract is found to be low activity in *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus* and *E. coli* species. EtOAc extracts show the highest activities on *Pseudomonas aeruginosa* and *E. coli*. But,EtOAc extracts also showed the medium activities on others microorganisms. In EtOH extract, exception of *Pseudomonas aeruginosa* showed the highest activities on tested microorganisms (Table 4).



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*



Bacillus pumilus *Candida albicans* *Escherichia coli*

Figure 5 Inhibition zones indicating the antimicrobial activities of various crude extracts of *P.betle*against test microorganisms

Table 4 Inhibition Zone Diameters of *P.betle*leaf Extracts

Microbial Strains	Inhibition Zone (mm)			
	PE	EtOAc	EtOH	H_2O
Bacillus subtilis	14(+)	19 (++)	22 (+++)	13 (+)
Staphylococcus aureus	13 (+)	19 (++)	20 (+++)	14 (+)
Pseudomonas aeruginosa	13 (+)	20 (+++)	17 (++)	16 (++)
Bacillus pumilus	12 (+)	19 (++)	20 (+++)	14 (+)
Candida albicans	13 (+)	19 (++)	22 (+++)	17 (++)
Escherichia coli	13 (+)	20 (+++)	24 (+++)	14 (+)

Agar well = 10 mm
(+) = 10-14 mm (low activity), (++) =15-19mm (moderate activity)
(+++) = 20 mm & above (high activity), (−) = no zone of inhibition

CONCLUSION

From this research work, the following inference could be deduced. *Piper betle*cultivation had been studied by treated of various concentrations of Chitosan Oligosaccharide fertilizer, such as 25 ppm, 50 ppm 75 ppm and not treated(control) ChitosanOligosaccharide fertilizer respectively. Number of leaves and yield were significantly increased in 25 ppm, 50 ppm and 75 ppm used of ChitosanOligosaccharide fertilizer. The yield of leaf after 135 days was found to be 6.02 kg in 50 ppm Chitosan Oligosaccharide used *P.betle*.

Relative mineral elements abundant percent, *P.betle*showed the richness of the mineral and Chitosan Oligosaccharide used *P.betle*leaf contain more mineral contents than control leaf. In the present investigation of phytochemical screening that there are several phytochemical constituents present in the various extracts of *P.betle*leaf. *P.betle*leaf found to contain alkaloids, carbohydrate, flavonoids, glycoside, organic acid, phenolic compound, quinone, saponin, starch, steroid, tannins and terpenoid. These phytochemical constituents are a good source of antimicrobial and antioxidant activity. Herbal medicines are valuable and readily available resources for primary health care.According to experimental results of this research, *P.betle*leaves contain various bioactive compound. From the results of in *vitro* antimicrobial activity assay, it may be concluded that ethyl acetate and ethanol extracts of *P.betle*leaf have more antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacilliuspumilus*and *Escherichia coli,* and antifungal activity against skin pathogen, *Candida albicans*.

Based on this study,cultivation of *Piper betle*usingChitosan Oligosaccharide fertilizer obtain more yield, more mineral contents and various extracts of leaves exhibited antibacterial activity against various gram positive and gram-negative pathogens.Therefore, Chitosan Oligosaccharide agriculture fertilizer can be used for substitute of chemical fertilizer to sustain without degrading the environment.

ACKNOWLEDGEMENT

We also wish to express our profound gratitude to Dr KyuKyuSwe, Professor and Head of Chemistry Department, Shwebo University for their encouragement and commitment for this research paper. We would like to express deep sense of gratitude to Dr Hlaing HlaingOo,Professor and Head (Reteired) and Dr SandarKhar, Professor and Head, Department of Chemistry, West Yangon University for their kind provision of the research facilities.

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