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Sardina Pilchardus Walb

Coastal Water of Bontang

Highlights

Barringtonia Swamp Forest

A Phytoassociation Analysis

Discovering Thoughts, Inventing Future

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By Sumit Manna, Parasuram Kamilya, Tushar Kanti Ghara & Anirban Roy

Abstract - For a long period, populations of *Helminthostachys zeylanica* (L.) Hook. have mostly been restricted in few geographic ranges due to alternation of their actual / potential habitat conditions. To find out the potential habitat of Helminthostachys zeylanica it is significant to identify their phytoassociates as well as the nature of association. Apart from the habitat and community analysis, present study also highlighted a strong positive correlation in association of *Helminthostachys zeylanica* with other co-existed plants of the forest. Present study depict some strong possibilities of true positive association between *Vetiveria zizanioides* (L.) Nash, *Barringtonia acutangula* (L.) Gaertn. and *Antidesma acidum* Retz., and also a strong negative correlation in association with *Eucalyptus globulus* Labill. Pearson correlation coefficient for interspecific covariance support there was a strong positive correlation in the density value for *Helminthostachys zeylanica* and *Barringtonia acutangula*. These findings depict the importance of presence of *Vetiveria zizanioides, Barringtonia acutangula* in the present habitat for establishment and increase of population size of *Helminthostachys zeylanica*.

Keywords : association, co-existence, conservation, covariance, phytoassociation, swamp.

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HELMINTHOSTACHYS ZEYLANICA IN BARRINGTONIA SWAMP FOREST A PHYTOASSOCIATION ANALYSIS

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Helminthostachys Zeylanica in *Barringtonia* Swamp Forest: A Phytoassociation Analysis

Sumit Manna [α], Parasuram Kamilya [σ], Tushar Kanti Ghara [ρ] & Anirban Roy [ω]

Abstract - For a long period, populations of *Helminthostachys zeylanica* (L.) Hook. have mostly been restricted in few geographic ranges due to alternation of their actual / potential habitat conditions. To find out the potential habitat of *Helminthostachys zeylanica* it is significant to identify their phytoassociates as well as the nature of association. Apart from the habitat and community analysis, present study also highlighted a strong positive correlation in association of *Helminthostachys zeylanica* with other co-existed plants of the forest. Present study depict some strong possibilities of true positive association between *Vetiveria zizanioides* (L.) Nash, *Barringtonia acutangula* (L.) Gaertn. and *Antidesma acidum* Retz., and also a strong negative correlation in association with *Eucalyptus globulus* Labill. Pearson correlation coefficient for interspecific covariance support there was a strong positive correlation in the density value for *Helminthostachys zeylanica* and *Barringtonia acutangula*. These findings depict the importance of presence of *Vetiveria zizanioides, Barringtonia acutangula* in the present habitat for establishment and increase of population size of *Helminthostachys zeylanica.*

Keywords : association, co-existence, conservation, covariance, phytoassociation, swamp.

I. Introduction

Helminthostachys zeylanica (L.) Hook. (commonly known as *Kamraj*) is a terrestrial, herbaceous fern allies belonging to the family Ophioglosaceae, distributed in the countries like China, India, Sri Lanka, Malay peninsula, New Guinea, Australia, Japan, Philippines, Solomon Island, New Caledonia (Clausen 1938) and Nepal (Chandra et al. 2008). In India they are mostly found in western forests of south (upto an elevation of 914.4 ft.) central India (Chandra et al. 2008), Bengal plains especially *Tarai* forests (Chakraborty et al. 2012), Assam, Uttarpradesh and Uttarakhand. Specificity in habitat preference and association pattern with other plants restricts this ancient gene pool to a certain geographical terrain. Royen (1963) reported *Helminthostachys zeylanica* as the undergrowth of *Barringtonia, Leptospermum* swamp of Papua intermingled with other herb associates like *Fimbristylis griffithii* Boeckeler, *Eriocaulon* sp. *Ilysanthes* sp. and *Xyris complanata* R.Br. and a number of epiphytes. In tropical countries like India, this novel species was found to be existed as the undergrowth of different forest composition like *Tectona grandis* L.f., *Eucalyptus globulus* Labill, *Mallothus philippensis* (Lam.) Mull. Arg., *Acacia catechu* (L.f.) Willd., *Dalbergia sissoo* Roxb. etc. especially at the shady, moist and humus rich areas (Joshi 2011). Sundari et al. (2012) found this monotypic genus to be grown in blackish soil with high water holding capacity shaded by *Cocos nucifera* L. It was also found to be grown in open grass swamps on the edge of Tarai forests especially along the blackish muddy soil of the river banks of Bahrachi, Uttarakhand, Gorakhpur (Dixit and Tripathi 1956) where the major dominant tree species found in 10 m. radius of *Helminthostachys zeylanica* was *Shorea robusta* C.F.Gaertn. admixed with *Eucalyptus* sp. *Terminalia belerica* Roxxb., *Syzygium cumini* (L.) Skeels, *Sygigium paniculata* Gaertn. Now a days, this evolutionary significant fern allies shrinked into few pockets of the world due to extensive grazing rate of the open grass land, extension of the cultivable land and over utilization by the local communities as food, fodder and mainly as medicine (Ghosh et al. 2004; Joshi 2011). Very low spore germination rate (Whittier 1987) and preference of vegetative propagation by the subterranean rhizomatous part also lead to confined this novel species to a certain localities. Habitat preference as well as specific association pattern with other plant associate regulates the key function to the establishment of this ancient genetic resource. Identification of their potential habitats and conservation of this species is to be needed immediately to protect this ancient gene pool. Phytoassociation analysis of *Helminthostachys zeylanica* with other plant species would be helpful to identify its strong positive and negative associates for facilitating to increase its population size through removal/keeping the associates.

II. Materials and Methods

a) Study Area

The studied *Barringtonia* forest *("Danga forest")* (25° 15' 200" N 88° 47' 913"E) was located at the transition between foot hills of the eastern Himalaya and the Gangetic plain. The soil is clay-loamy because of submergence of the area with over flowed water from near by canal during monsoon for at least four months of the year. Maximum annual rainfall of this area is 1921

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mm. and the temperature varies from 7° C to 39° C. *Barringtonia acutangula* (L.) Gaertn., *Eucalyptus globulus* Labill. and *Antidesma acidum* Retz. were the only tree species co-occurred with *Helminthostachys zeylanica* at this swampy grass land with a ground cover of *Vetiveria zizanioides* (L.) Nash. Forest at the north-western side of *Helminthostachys zeylanica* population comprised of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn, *Shorea robusta* C.F. Gaertn., *Litsea glutinosa* (Lour.) C. B. Rob., *Syzygium cumini, Holarrhena antidysenterica* (L.) Wall. and others (Fig. 1). There was a trend of agricultural expansion at the south and eastern side and presence of human settlement at the north western side of the swamp (Fig. 1). Water stagnant at the middle (323.74 Sq. m.) of this 2428.12 Sq. m. study area through out the year due to lower elevation. At the southern side of this water body large number of *Barringtonia acutangula* were present where as the northern side no other tree species were existed except a few individuals of *Eucalyptus globulus*. (Plantation by the forest department) and few cut stamp of *Barringtonia acutangula*. (Fig. 1).

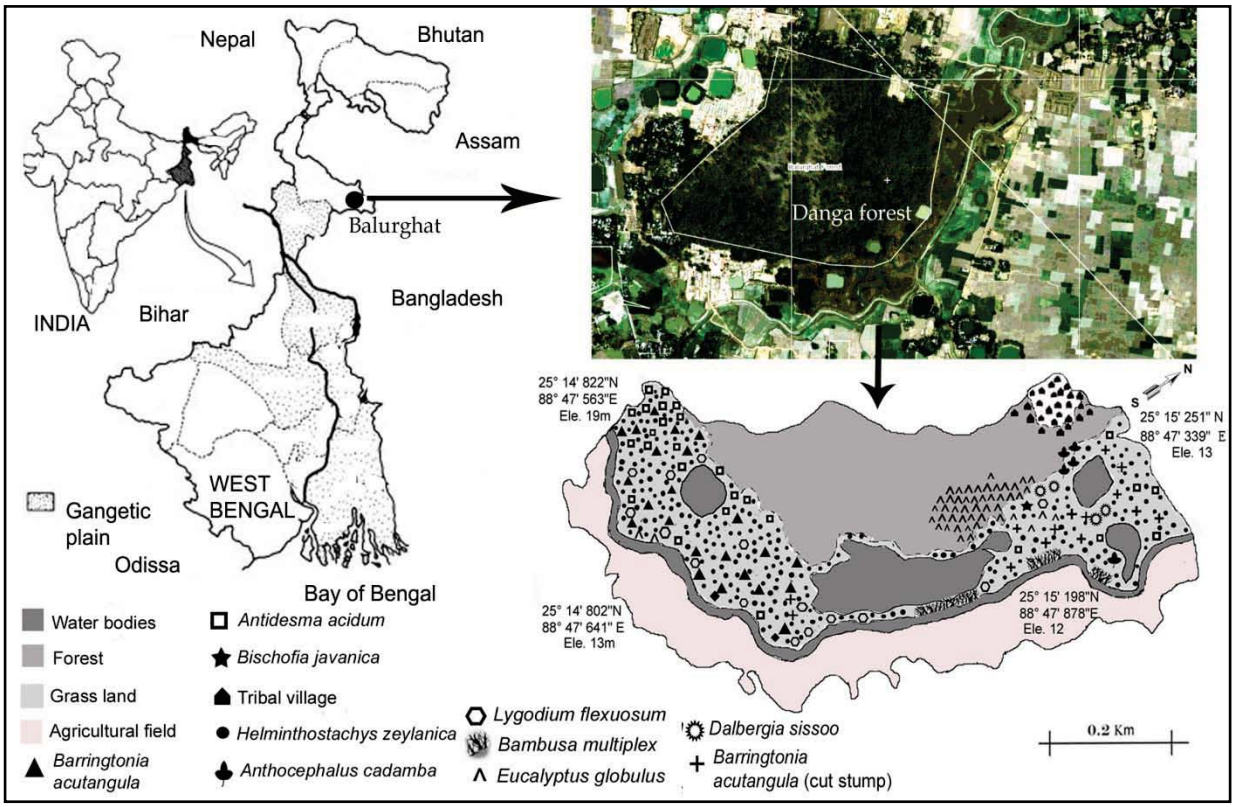

Figure 1 : Vegetation map of *Helminthostachys zeylanica* and other plant community in *Barringtonia* swamp

b) Methods

The study area was divided into two sites based on the variable habitat condition of *Helminthostachys zeylanica*. Site -1 (25° 15' 251" N 88° 47' 339"E) was an open grass land mostly covered with *Vetiveria zizanioides* along with some noted *Barringtonia acutangula* cut stumps and a few planted *Eucalyptus globulus* at its extreme western side and the tribal settlement with two ponds at its northern part. Site-2 (25° 14' 802"N 88° 47' 641" E) was densely covered with *Vetiveria zizanioides* along with many arborsent *Barringtonia acutangula* and *Antidesma acidum.*

For community analysis, sixteen sampling units (SUs) (Quadrats of 20.88 Sq.m. sizes) were plotted separately on both study sites (Site -1 and 2). Quadrats are plotted randomly in each site to avoid biased sampling. Structural Parameters like Density (D), Abundance (A), Relative Density (RD), Relative Frequency (RF) and Importance Value Index (IVI) were estimated by using standard procedure (Gopikumar et al. 2005; Magurran 1988; Manna et al. 2012). To study the inter-specific association (if any) existed between *Helminthostachys zeylanica* and other 6 associated plant species present in this swampy forest plant community, 2×2 contingency / species association table was prepared separately from presence-absence data matrix (generated from 16 SUs plotted in each site) for pair wise comparison between them both in site-1 and 2 separately (Ludwig and Reynolds 1988; Manna et al. 2012). Large numbers of sampling units (16 in each study site) were taken to avoid biased chi-square values as much as possible (Eq. 1). For further continuity,

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correction to ensure a closer approximation to the theoretical continuous Chi-square distribution, Yates's correction formula (Eq. 2) was adopted. Association was measured by computing Ochiai *(OI),* Dice *(DI)* and Jaccard *(JI)* Index (Eq. 3, 4 & 5) (Ludwig and Reynolds 1988). To study the interspecific co-variation (if any) existed between *Helminthostachys zeylanica* and other 6 plant associates present in the community, Pearson's Correlation coefficient (Eq. 6) was calculated based on abundance data of *Helminthostachys zeylanica* and other phytoassociates (Ludwig and Reynolds 1988). Abundance data were derived from the data of 32 Quadrats plotted through out the study area.

$$\chi_t^2 = \frac{N(ad-bc)^2}{mnrs} \quad (1)$$

$$\chi_t^2 = \frac{N\left[|(ad)-(bc)|-\left(N/2\right)\right]^2}{mnrs} \quad (2)$$

$$DI = \frac{2a}{2a+b+c} \quad (3)$$

$$JI = \frac{a}{a+b+c} \quad (4)$$

$$OI = \frac{a}{\sqrt{a+b}\,\sqrt{a+c}} \quad (5)$$

$$r(i,k) = \frac{\sum_{j=1}^{N} Y_{ij}Y_{kj} - \{(\sum_{j=1}^{N} Y_{ij})(\sum_{j=1}^{N} Y_{kj})/N\}}{\sqrt{\left[\sum_{j=1}^{N} Y_{ij}^2 - \{(\sum_{j=1}^{N} Y_{ij})^2/N\}\right]\left[\sum_{j=1}^{N} Y_{kj}^2 - \{(\sum_{j=1}^{N} Y_{kj})^2/N\}\right]}} \quad (6)$$

a= the number of SUs where both species occur, b = the number of SUs where species A occurs but not B, c = the number of SUs where species B occurs but not A, d = the number of SUs where neither A nor B are found and N = the total number of SUs (N = a+b+c+d), Y_{ij} =the abundance of ith species in j^{th} SU, Y_{kj} = the abundance of the kth species in the jth SU.

As *Vetiveria zizanioides* population was densely matted in the entire studied forest site during the growing season of *Helminthostachys zeylanica,* the structural parameters including the data on its abundance had not been calculated and thus excluded from Pearson's Correlation coefficient test.

III. Results

Helminthostachys zeylanica was found to be co-existed with *Vetiveria zizanioides, Barringtonia acutangula, Antidesma acidum*, *Eucalyptus globulus*, *Lygodium flexuosum* (L.) Sw. Ab, F%, RD, RF and IVI values of *Helminthostachys zeylanica* was comparatively higher in Site -2 than Site -1 (Table 1 and Fig. 2). In every year during monsoon the whole study area was inundated by the overflow of near by canal for a period of 5 to 6 months. On receding water, the entire area was an optimum habitat for *Vetiveria zizanioides*, *Helminthostachys zeylanica* and germination ground of *Antidesma acidum* as *Antidesma acidum* trees were distributed at higher elevation of this marshy land where in was not in submergence during monsoon. During summer 2-3 spikes were developed at tip of each individual of *Helminthostachys zeylanica* and most of them were noticed to be bifurcated from the base (Fig. 3) which is a very unusual phenomenon (Manickam and Irudayaraj 1994). It was observed that all the individuals were developed from upper surface of subterranean horizontal rhizome and not a single soul found isolated inspite of its ample spore production. Interspecific association indices between *Helminthostachys zeylanica* and other co-existing plants (Table 2 and Fig. 4) indicated that *Helminthostachys zeylanica* had maximum positive association with *Vetiveria zizanioides* followed by *Barringtonia acutangula* and *Antidesma acidum* where as strong negative association was found with *Eucalyptus globulus* (for all species we accept 5% probability level). Pearson correlation coefficient for interspecific covariance indicated that there was a strong positive correlation in the density value for *Helminthostachys zeylanica* and *Barringtonia acutangula* (at 5% probability level) (Table 3).

Table 1 : Structural parameter of the *Helminthostachys zeylanica* and other plant community

Plant Species	Density		Abundance		Frequency %		Relative density		Relative frequency		Importance value index	
	S. 1	S. 2	S. 1	S. 2	S. 1	S. 2	S. 1	S. 2	S. 1	S. 2	S. 1	S. 2
H. zeylanica	0.665	2.610	17.077	54.500	81.250	100.000	17.317	68.019	19.697	24.242	37.014	92.261
B. acutangula	0.003	0.069	1.000	2.091	6.250	68.750	0.078	1.794	1.515	16.667	1.593	18.461
B. acutangula (Cut stump)	0.021	0.003	3.500	1.000	12.500	6.250	0.546	0.078	3.030	1.515	3.576	1.593
E. globulus	0.009	0.003	1.000	1.000	18.750	6.250	0.234	0.078	4.545	1.515	4.779	1.593
L. flexuosum	0.009	0.003	1.000	1.000	18.750	6.250	0.234	0.078	4.545	1.515	4.779	1.593
A. acidum	0.003	0.440	1.000	11.308	6.250	81.250	0.078	11.466	1.515	19.697	1.593	31.163

S: Site.

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Table 2 : Inter specific association indices and test statistics between *Helminthostachys zeylanica* and other plant species in site 1 and 2

Site-1 Sp. Pair	AT	Chi-Square	Yate's Chi-square	Association Index			Site-2 Sp. Pair	AT	Chi-Square	Yate's Chi-square	Association Index		
				OI	*DI*	*JI*					*OI*	*DI*	*JI*
1--2	+	0.25	0.68	0.28	0.14	0.08	1--2	+	4.62*	0.68	0.9	0.9	0.87
1--3	+	0.53	0.06	0.39	0.27	0.15	1--3	+	0.07	3.48	0.3	0.1	0.07
1--4	+	1.47	0.06	0.89	0.89	0.8	1--4	+	7.47*	1.37	1.0	1.0	0.93
1--5	_	0.52	0.01	0.32	0.25	0.14	1--5	_	16*	3.48	0.0	0.0	0.00
1--6	+	0.85	0.01	0.48	0.38	0.23	1--6	+	0.07	3.48	0.3	0.1	0.07
1--7	+	0.25	0.68	0.28	0.14	0.08	1--7	+	4.62*	0.68	0.9	0.9	0.87

*For association between 1-2, 1-4 and 1-7 we accepted 5% probability level

AT: Association type; Ochiai: *OI;* Dice: *DI;* and Jaccard: *JI;* SU: sampling unit; Species 1: *Helminthostachys zeylanica;* species 2: *Barringtonia acutangula;* species 3: *Barringtonia acutangula* (cut stump); species 4: *Vetiveria zizanioides;* species 5: *Eucalyptus globulus;* species 6: *Lygodium flexuosum;* species 7: *Antidesma acidum.*

Table 3 : Interspecific covariation showing Pearson's correlation coefficient

Species Abundance	SU1	SU2	SU3	SU4	SU5	SU6	Species Pair	PCC
1	79.8	47.6	43.2	22.25	17.8	10.33	1--2	0.83*
2	2	2	2.25	0	0	0		
5	1	1	0	0	17.4	9	1-5	-0.50
7	0	0	0	5	0	0	1-7	0.51

*For abundance correlation between species 1-2 we accept 5% probability level

Pearson's correlation coefficient: PCC; Sampling unit: SU; Species 1: *Helminthostachys zeylanica;* species 2: *Barringtonia acutangula;* species 5: *Eucalyptus globulus;* species 7: *Antidesma acidum.*

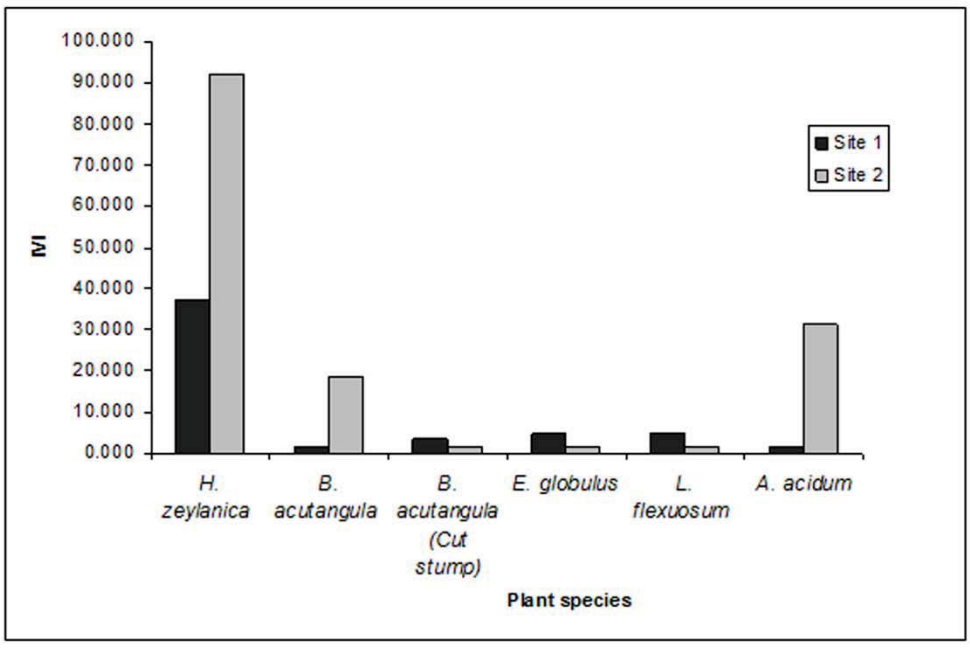

Figure 2 : Comparative account of Important Value Index of different plants in the two study sites

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Figure 3 : *Helminthostachys zeylanica* bearing spike bifurcated from the base

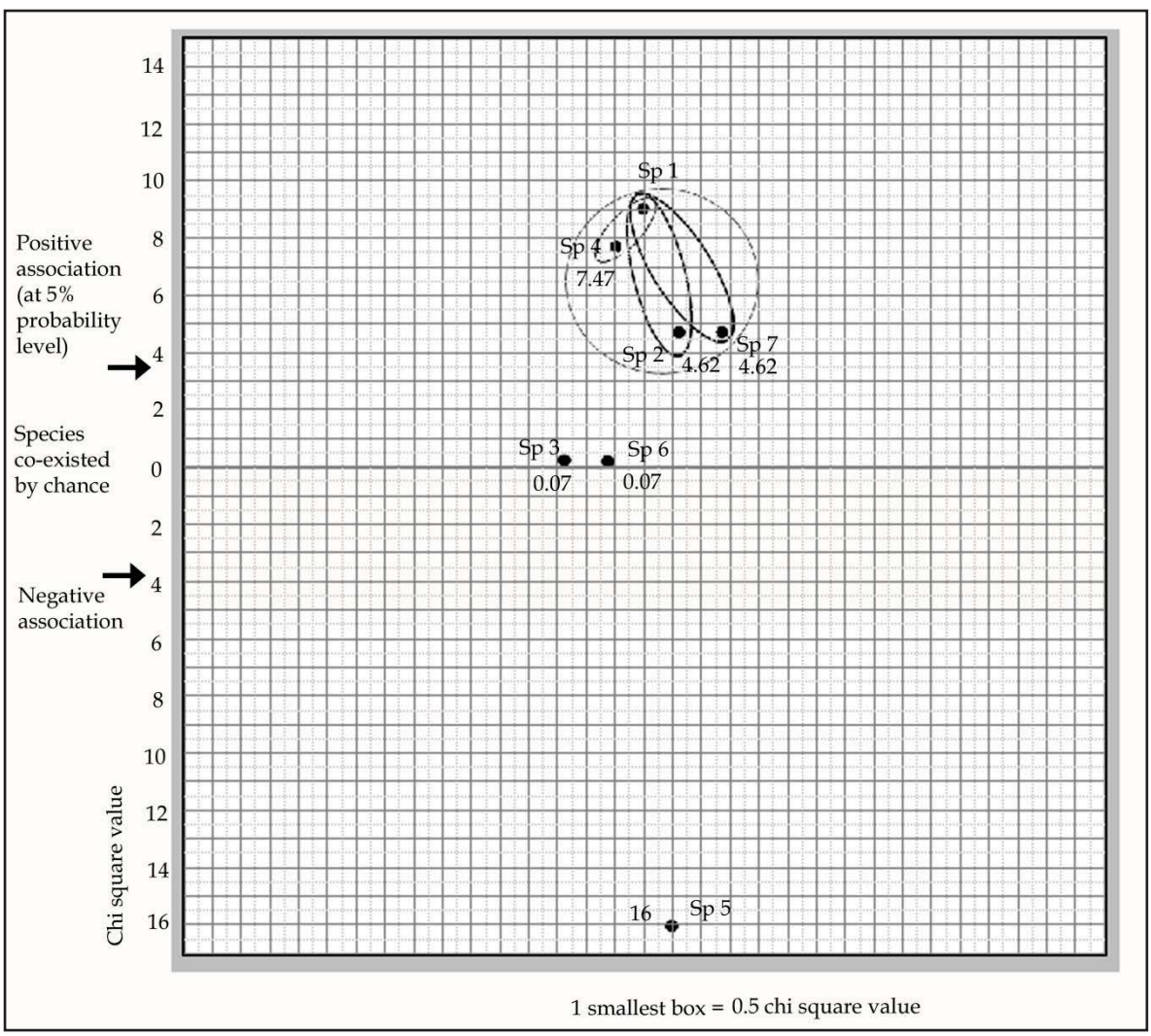

Figure 4 : Plexus diagram showing pattern of association between *Helminthostachys zeylanica* and others

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IV. Discussion

Less disturbance, moist shady condition by the canopy of large *Barringtonia acutangula* trees and claiy humus rich substratum covered by dense population of *Vetiveria zizanioides* created a fabulous microclimate for better population size of *Helminthostachys zeylanica* with maximum Ab, F%, RD, RF in site-2 unlike site-1 (Joshi 2011; Sundari et al. 2012). Yearly monsoon sedimentation in the habitat is also helpful for its successful establishment (Goswami 2007). Presence of *Vetiveria zizanioides* was supposed to be playing one of the key role to the establishment of *Helminthostachys zeylanica* in this habitat. *Helminthostachys zeylanica* had its luxuriant growth because rhizome of this species remained entangled with the tuft of rigid aggregate roots of *Vetiveria zizanioides* resisting from monsoon flash rather accumulating excess sediment. Interspecific association indices supported that there was a strong positive association existsted between *Helminthostachys zeylanica* and *Vetiveria zizanioides* in the present habitat. This also supports their co-operative and similar mode of response to the different environmental factors. Strong positive association of *Helminthostachys zeylanica* with *Barringtonia acutangula* as well as strong positive correlation in the density value (from Pearson's Correlation coefficient) of these two species also indicated the importance of *Barringtonia acutangula* to the establishment and formation of better population size of *Helminthostachys zeylanica* in this low land swampy plant community. The present finding was also supported by the observation of Royn (1963) who reported *Helminthostachys zeylanica* population in the *Barringtonia- Leptospermum* swamp in Papua. *Antidesma acidum* was also thought to be performed the same function as in *Barringtonia acutangula*. Strong negative association of *Eucalyptus globulus* with *Helminthostachys zeylanica* suggested the population of *Helminthostachys zeylanica* is declining with the increasing individuals of planted *Eucalyptus globulus* which contradicts the observation of Joshi (2011) based on qualitative study.

Helminthostachys zeylanica is very sensitive to the different environmental factors like temperature, humidity, water availability, wind velocity etc. and can manifest characters in a different way than that was determined genetically (Sundari et al. 2012). Presence of both branched and unbranched spike in the same study site may due to the same reason.

The individuals of *Helminthostachys zeylanica* are mostly ramets as developed from the rhizome, still they produce huge amount of spores which are either nonviable in nature or there is an sexual incompatibility within the gametophyte that hinders the development of genets. Inspite of vegetative propagation huge amount of energy investment in *Helminthostachys zeylanica* for spore production is still in question, but may be hypothesized that the shifting sexual mode from existed vegetative reproduction during unfavourable or other environmental condition may lead their existence.

V. Conclusion

Besides natural calamities, the anthropogenic pressure like deforestation, alternation of habitat etc. causes serious threats to the extinction of numerous ancient evolutionary significant novel plants. *Helminthostachys zeylanica,* being an ancient (Devonian) land vascular plant and keeping transitional status of true fern and fern allies is significantly under serious threat all over the world. Present findings will help to protect this ancient gene pool through keeping the positively associated plants like *Barringtonia acutangula, Vetiveria zizanioides, Antidesma acidum* and removing the negatively associated plants like *Eucalyptus globulus* from the habitat of *Helminthostachys zeylanica* to get better population size during in situ or ex situ conservation programme in future.

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Investigation on Fish Assemblages around Cooling Water System Outlet in the Coastal Water of Bontang City, East Kalimantan

By Iwan Suyatna & Ahmad Syafei Sidik

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Keywords : cooling water system outlet, tide, water temperature, salinity, bontang city.

GJSFR-C Classification : FOR Code: 291204

INVESTIGATION ON FISH ASSEMBLAGES AROUND COOLING WATER SYSTEM OUTLET IN THE COASTAL WATER OF BONTANG CITY, EAST KALIMANTAN

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Iwan Suyatna [α] & Ahmad Syafei Sidik [σ]

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I. Introduction

Bontang City geographically stretches between 117°23' and 117°38' E and 0°01' and 0°12' N, and lies between Mahakam Delta and Sangkulirang Bay, East Kalimantan (Figure 1). Defined as a centre for petrochemical industries receiving raw gas materials by piping from oil and gas exploitation in the Mahakam Delta, the coastal zone of Bontang City continuously transforms with new constructions such as industrial plants, reclamation, ports, and channel dredging. Newell (1998) reported that dredging and land reclamation disturb benthic communities, and as much as 30% of total fisheries yield to man is derived from benthic resources including demersal fishes. The development of ports and fisheries harbours also involve dredging and disposal activities to maintain the required depths for navigation (Kudale 2010, Commision 2007 and Commission, 2009). Some coastal water areas in Bontang City accomodate waste hot water released from cooling water system outlets of some chemical companies. This heated water carrying much thermal energy may greatly affect on physical, chemical and biological characteristics of water. Hot water flowing from the outlets is already subject to the rising of sea water temperature in Bontang City and able to harm fishes. Since very limited information is available on fish living around the areas, fish species and their asssemblages are urged to investigate.

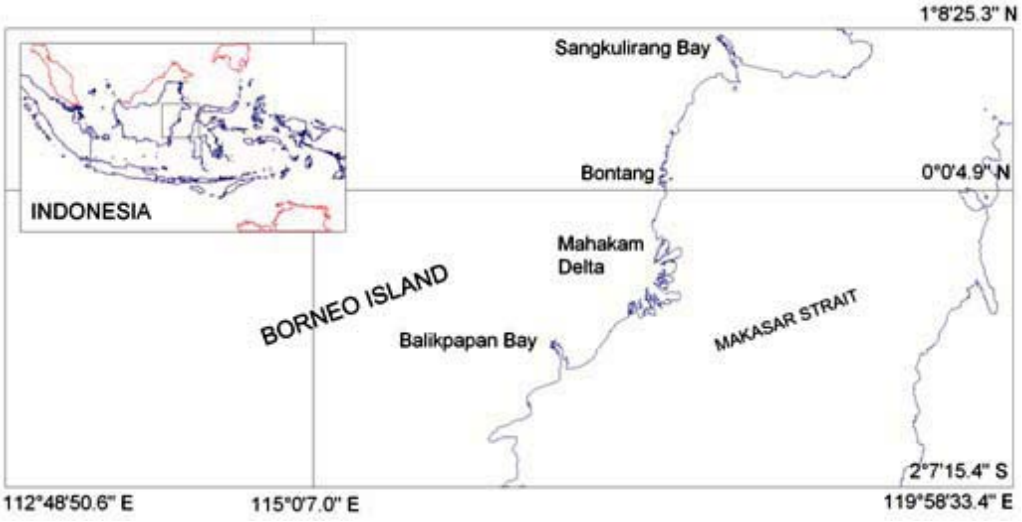

Figure 1 : Map showing location for the investigation of fish species and their assemblages in Bontang City, East Kalimantan

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The investigation aimed at identifying fish species and studying fish community structure and their assemblages in waters around the outlets. Further, fishes sampled from the mouth of outlet and inlet were also observed.

II. Materials and Methods

The investigation was undertaken in January and February 2013. Fishes samples were collected from water around the outlet of a methanol industry (PT Kaltim Methanol Industri) in Bontang City. The water is a dredged channel with approximately 400 m width and 10 m depth. In January 13th a bottom minitrawler sizing 11 m x 1.25 m equipped with a net of 12 m long having mesh size of 1.25 inches at front and 1.5 m long as cod end having mesh size of 1.0 inch at end was used to perform fish sampling in spring tide. Another fish sampling was conducted in neap tide in the following month on February 5th. Limited towing time 15 to 20 minutes was decided to avoid net damage due to sharp hard substrate. Samplings were done between 150 m going west and 200 m going east from the outlet. Geographic positions of net setting and hauling were recorded by GPS Garmin 60 CSx. Fish samples were also taken at the mouth of outlet and water intake by angling using hook #7 and nylon line 0.33 mm. Live bait 3 to 4 cm of small shrimps *Metapenaeus* sp was used. To obtain the data of sea surface tempetaure and salinity in sampling area, three 500 m line transects having eight to 11 observed points each were constructed with certain angle from the outlet. The interval distance between observed point was 50 m. Sea surface temperature and salinity were measured using water checker Horiba U-50 series.

During investigation, hydrographic aspects such as tide and water depth including water current were surveyed using tidal scale pole, echosounder GPSmap 2108 Garmin and Braystoke BFM001 current meter respectively. Tide and tidal water current were observed every 30 minutes in spring tide (Januari 12th and 14 th to 15 th) and in neap tide (February 4 th to 6 th). Sounding water depth was conducted on January 12th. Data of sea surface temperature and salinity were analyzed by descriptive statistics using SPSS version 15. Software of PAlaeontological STatistics PAST version 3 was also used to describe fish community structure through index of diversity including dominancy and Margalef richness. Catch per unit effort CPUE was used to obtain fish density (Can *et al.*, 2005). Fish species were identified according to Anam and Mostarda (2012), Peristiwady (2006), Allen (2000), and Masuda et al. (1975), while fish sample photographs were documented using digital camera of Nikon Coolpix AW100.

III. Results and Discussion

a) Environmental Factors

Since physical, chemical dan hydrographic conditions in waters may influence a distribution pattern of fish (Hsieh, 2012), some environmental factors measured during investigation in the water around outlet are described in Table 1.

Table 1 : Sea surface water temperature and salinity measured in water around the outlet of a methanol industry in Bontang City, East Kalimantan

	Temperature (°C)			Salinity (°/oo)		
	Spring tide		Neap tide	Spring tide		Neap tide
	HWL	LWL		HWL	LWL	
Numer of data (n)	29	29	29	29	29	29
Distribution	32 to 40	31 to 41	32 to 40	32 to 35	31 to 34	28 to 31
Frequency	36 (12)	35 (10)	34 (9)	34 (20)	32 (15)	30 (18)
Range	8	10	8	3	3	3
Mean	35.76	34.69	34.66	33.86	32.31	30.14
Variance	3.19	4.29	2.73	0.98	0.51	0.48
Standard Deviation	1.79	2.07	1.65	0.99	0.71	0.69

Notes : HWL= high water level, LWL= low water level. Number in parenthesis shows the frequency.

Sea surface water temperature in spring tide both at HWL and LWL tended to be fluctuated as shown in the above table. The most frequency of sea water temperature during the investigation occurred at 35 to 36°C. However, the water in the investigation area in general exhibited marked extreme water temperature exceeding 5°C of normal limit, while in neap tide it appeared approximately 34°C. The rise and fall of sea surface monitored for about 73.5 hours during spring tide and 50 hours during neap tide showed a difference pattern (Figure 2). During neap tide, the HWL and LWL were occurring almost at similar level in several days. This tide enabled sea water to mix more with the water from the outlet and lead to sea water temperature degradation. Theoritically, young fish can survive in water 2°C warmer than the maximum temperature adults

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can tolerate (Moyle and Cech, 2000). While Steady et al. (2011) suggests the maximum allowable temperature uplift is between +2°C and +3°C.

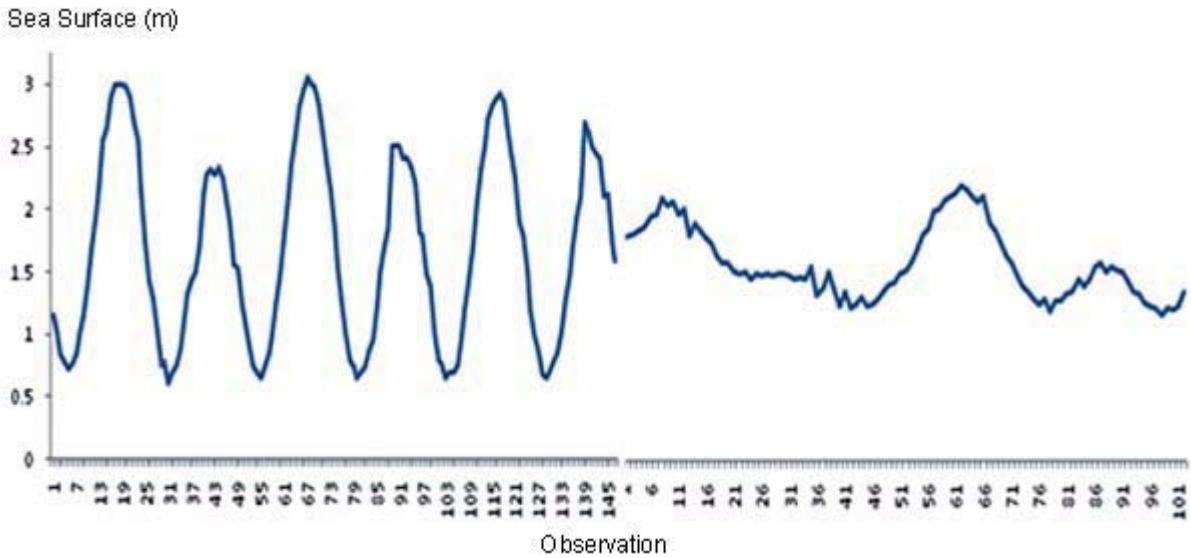

Figure 2 : The rise and fall of sea surface at the investigation site during spring and neap tide (left and right) in Bontang City, East Kalimantan

Salinity distribution in spring and neap tide ranged from 31 to 35⁰/oo and 28 to 31⁰/oo respectively. The salinity of 34⁰/oo was the most concentration showing within the normal limit. The salinity concentration in neap tide was much more less than in spring tide. During the investigation, the area was rained twice and predicted to have affected to salinity. Hydrographic surveying without measuring tides directly is one of many uses (Federation Internationale Geometre, 2013). For the fish species investigation purpose, the survey was carried out with directly measuring tides. It obtained 1,158 water depth points and the deepest water depth measured was 13.7 m. Using the tidal range as a correction factor, the water depth for three levels based on tidal cycles is presented in the following table.

Table 2 : Water depths at the investigation site measured during hydrographic survey based on tidal cycles

Vertical Surface (m)	Tidal Cycle		Δd
	Spring Tide	Neap Tide	
Range of tidal level	-2.05	-1.03	-1.02
High Water Level	-14.28	-13.06	-1.22
Low Water Level	-12.03	-12.03	0.00
Mean Water Level	-13.05	-12.55	-0.50

As it is very important to fish and related to fish distribution, water current was observed at three water columns accordingly. The result of measurement is presented in Table 3 below.

Table 3 : Ranges of water currents (m s^{-1}) measured at three water columns in investigation site

	Surface Water (0.2d)	Mid Water (0.6d)	Bottom Water (0.8d)
Observation Hour	Spring Tide (Jan 12 and 15, 2013)		
10 am to 18.30 pm	0.031 to 0.086 (n=18)	0.031 to 0.149 (n=18)	0.038 to 0.101 (n=18)
00 am to 08.30 am	0.031 to 0.081 (n=17)	0.028 to 0.101 (n=17)	0.031 to 0.081 (n=17)
	Neap Tide (Feb 04 and 05, 2013)		
9.30 am to 18.30 pm	0.041 to 0.101 (n=18)	0.033 to 0.081 (n=18)	0.036 to 0.068 (n=18)
00.00 am to 16.00 pm	0.033 to o.081 (n=33)	o.036 to 0.091 (n=33)	0.033 to 0.058 (n=33)

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A recent investigation on hydrographic aspects of fish trap "Julu" fisheries using a tidal water current, the maximum during spring and neap tide was observed 1.48 and 0.78 m s^{-1} respectively (Suyatna, et al., 2012). Sepa (2006) identified that tidal current of 0.70 m s^{-1} belongs to a strong current. Thus the water currents measured at investigation site were categorized as weak currents. It was probably because the site physically includes as a semi-closed water.

b) Number of Species, Composition and Fish Densities

During investigation, a total of 5670 fishes was caught from the water around the outlet of a methanol industry. Of all fishes, 5164 individuals resulted from bottom minitrawl sampling consisted of 40 different fish species and 19 families. The other 560 individuals caught with angling covering 37 different species belonging to 12 families (Table 4 and 5).

Table 4 : List of fishes caught from all bottom minitrawl samplings during spring and neap tide

Common name	Species name	Samp size	Spec num	Length (cm) Min	Length (cm) Max	Weight (g) Min	Weight (g) Max
Snappers (Lutjanidae)	: *Lutjanus* sp	5	4	4	11.2	1.1	21.9
Groupers (Serranidae)	: *Epinephelus* sp	3	3	10.5	29	16.3	306.6
	: *Cephalopholis* sp	7	2	4	18.5	2	76.3
Rabbitfish (Siganidae)	: -						
Surgeonfish (Acunthuridae)	: -						
Spotted Scats (Scatophagidae)	: -						
Sea Breams (Nemipteridae)	: *Nemipterus* sp	64	1	6.9	20.5	3.6	139.5
	:*Pentapodus* sp	2	2	7		12	
Emperors (Lethridae)	: -						
Damselfish (Pomacentridae)	: -						
Trevallies (Carangidae)	: *Carangoides* sp	1	1	7		5.4	
	: *Alectes* sp	2	1	2.5	6	0.6	4.1
Queenfish (Carangidae)	: *Scomberoides* sp	2	1	4.9	10	0.4	6.7
Herring (Elopidae)	:-						
Fusilier (Caesionidae)	: -						
Silver Biddy (Gerreidae)	: *Gerres* sp	105	3	6	19.4	1.5	54.1
Ponyfishes (Leiognathidae)	:	4752					
	: *Leiognathus* sp	1409	3	3,0	14.3	0.2	35.7
	: *Gazza* sp	3170	2	4	8	0.9	8.9
	: *Secutor* sp	173	2	6.2	10	2.8	11.1
Goatfishes (Mullidae)	: *Upeneus* sp	121	2	5.4	13	0.9	13.9
Lizardfish (Harpodontidae)	: *Saurida* sp	28	1	6.5	25.6	1,0	134.9
Flathead (Platycephalidae)	: *Platycephalus* sp	14	1	8	31	2.1	200.3
Perchlet (Chandidae)	: *Ambassis* sp	2	1	5	6	0.9	1.8
Pufferfish (Tetraodontidae)	: *Arothron* sp	7	1	4.5	18.5	1.6	122.2
Cardinalfish (Apogonidae)	: *Apogon* sp	16	1	5.5	9	1.6	5.5
Cutlassfish (Trichiuridae)	: *Trichiurus* sp	4	1	15.7	81,0	10,0	516.8
Flounder (Bothidae)	: *Pseudorhombus* sp	19	1	5.2	6.5	0.5	5.9
Anchovy (Engraulidae)	: *Stolephorus* sp	1	1	8		3	
Razorfish (Centriscidae)	: *Centriscus* sp	1	1	6.5		0.3	
Northern Pilchard (Clupeidae)	: *Amblygaster* sp	5	2	8.1	10	7.1	10.8
Ray (Dasyatidae)	: *Dasyatis* sp	2	1	10	51	9.1	516.4
Scorpionfish (Scorphaenidae)	: *Pterois* sp	1	1	11.5		14.9	
		5164	40				

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Table 4 shows in majority the size distribution of fish indicated juvenile stage and larvae such as trevallies (*Carangoides* sp, *Alectis* sp), groupers (*Epinephelus* sp, *Cephalopholis* sp) and snappers (*Lutjanidae* sp). These fishes nursed and fed in the water around the outlet of investigation site. Blaber (2000) stated that estuaries and shallow waters throughout the world play a major role as nursery areas for a wide variety of organisms. *Leiognathus* sp, *Secutor* sp and *Gazza* sp belonging to the family Leiognathidae were recorded as the most abundant species (Figure 3), followed by Goatfish *Upeneus* sp (Mullidae), Silver biddy *Gerres* sp (Gerreidae) and Sea breams *Nemipterus* sp (Nemipteridae). These fishes were categorized as demersal fishes and having wide distribution on the basis of depth (Suyatna et al, 2010). According to the diversity index, dominance species occured at LWL of spring tide. While Shannon index showed various fish species appeared at HWL of both spring and neap tide. Species richness Margalef increased at HWL of spring and neap tide (Table 6).

Table 5 : List of fish groups caught by angling from mouth of the outlet and intake water of methanol industry

Fish group		Sample size	Species number	Length weight distribution			
				Length		Weight	
Common name	Species name			Min	Max	Min	Max
Snappers (Lutjanidae)	: Lutjanus sp	252	12	9.2	28	13.5	207
Groupers (Serranidae)	: Epinephelus sp	1	1	14	-	39.5	-
	Cephalopolis sp	7	2	12.1	20	26.2	141
Rabbitfish (Siganidae)	: Siganus sp	3	3	21.5	-	179.5	-
Surgeonfish (Acanthuridae)	: Acunthurus sp	2	1	31	-	589.8	-
Spotted scat (Scatophagidae)	: Scatophagus sp	1	1	16.5	-	159	-
Sea Breams (Nemipteridae)	: Nemipterus sp	9	1	12.5	19.6	27.4	115
	Scolopsis sp	26	2	9	18	11	46
	Pentapodus sp	28	3	6	18.5	2.6	18
Emperors (Lethrinidae)	: Lethrinus sp	26	2	8.7	21.5	12.5	162
Damselfish (Pomacentridae)	: Abudefduf sp	4	1	13.5	16.5	47.3	100
Trevalies (Carangidae)	: Carangoides sp	83	4	15	26.6	48.5	321.3
	Caranx sp	53	1	14	25	41.6	197.6
Herring (Elopidae)	: Megalops sp	2	1	20	41	231.8	590.8
Fusilier (Caesionidae)	: Caesio sp	12	1	16.2	23	53.8	10
Silver biddy (Gerreidae)	: Gerres sp	2	1	15	-	56.6	-
		510	37				

Table 6 : Indices of diversity, dominance and margalef richness of fish sampled during spring and neap tide in water around the outlet

	Spring tide						Neap tide Sampling		
	Sampling at HWL			Sampling at LWL					
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Taxa	22	16	13	10	16	11	15	18	12
Individuals	1108	130	2749	364	530	284	30	101	112
Shannon	1.101	2.14	0.9354	0.5371	0.6871	0.8282	2.478	1.569	1.623
Dominance	0.5915	0.1651	0.4577	0.8039	0.7557	0.6552	0.1022	0.3387	0.2709
Margalef	2.996	3.082	1.515	1.526	2.391	1.77	4.116	3.684	2.331

Angling at mouth of the outlet and water intake yielded the number of different fish species as mentionned in the Table 5. Five of 12 families consisting of Siganidae, Acanthuridae, Scatophagidae, Elopidae and Caesionidae were not observed from all samples taken by minitrawl. Acanthuridae as herbivorous fishes

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(Navaro and Vivien, 1981) resembles the deeper bodied siganids in general shape and often occur together. Members of Scatophagidae are the euryhaline fishes commonly found in estuaries and harbours. Elopidae and Caesionidae commonly distributed in coastal waters, members of Elopidae are sometimes travelling in schools in open water (Matsunuma, 2011). The most common fish species caught at water intake was the member of Lutjanidae, *Lutjanus* sp composed of 12 species and family Carangidae i.e *Carangoides sp* and *Caranx sp* (Figure 4 and 5). Meanwhile, members of Nemepteridae such as *Pentapodus* sp were easy to fish at mouth of hot water outlet (± 40°C) very close to the outlet (Figure 4). These species are common inhabitants of sand-rubble bottoms near coral and rocky reefs (Allen and Erdmann, 2009).

Figure 3 : Members of family Leiognathidae observed during investigation. At left *Leiognathus fasciatus,* First row from left *Gazza minuta, L. splendens, L. nuchalis*. Second row from left *G. minuta, Secutor ruconius, S. indicus* (All photos were taken at site)

Figure 4 : Nine of twelve members of family Lutjanidae observed during investigation. First row from left *Lutjanus decussatus, L. johnii, L. fulvus,* Second row from left *L. lineolatus, L. ruselli, L. spilurus,* Third row *L. vitta, L. fulviflamma, L. rufolineatus* (All photos were taken at site)

Figure 5 : Members of family Nemipteridae observed at mouth of the outlet. From left *Pentapodus setosus, Scolopsis ciliatus, P bifasciatus* (All photos were taken at site)

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Figure 6 : Members of family Serranidae and Carangidae observed at water intake. First row from left *Epinephelus coioides, Cephalopholis polycentron, E. Rivulatus*. Second row from left *C. sonnerati, E. corallicola, Carangoides ferdau*. Third row *Carangoides bajad, Caranx sexfasciatus, Carangoides coeruleopinnatus*
(All photos were taken at site)

Acoording to the analysis of CPUE, the significant fish density was dominated by four families in which members of family Leiognathidae showing the most populated fish (Table 6). Suyatna et al (2000) found out that Leiognathidae was the most important group of fish in Mahakam Delta.

Table 6 : Significant fish density (ind/km^2) in water around the outlet based on family and tidal cycle

	Spring tide		Neap tide
	HWL	LWL	
Leiognatidae	328356	99200	16534
Mullidae	9067	1688	0
Gerreidae	6756	1689	889
Nemipteridae	4356	622	711
Apogonidae	978	178	267

As seen in the above table, fish density was greatly affected by tidal cycle, and fishes tended to enter the water around the outlet together with sea rise. At low tide and neap tide fish density decreased.

IV. Conclusion

The assemblage of fish species in water around the outlet, at mouth and inlet differed. During the investigation, 57 different species were identified from 5670 fishes. Members of family Leiognathidae were the most common fish species observed in water around the outlet. While members of family Lutjanidae were assemblaged at water intake and certain species of Nemipteridae seemed to withstand temperature pressure. Fishes assemblage was greatly influenced by tidal cycle. Highest shannon and margalef index occurred in neap tide, while highest dominance index was observed at LWL in spring tide. The water condition around the outlet was included as relatively good indicated by the number and species of fish caught.

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Estimation De La Croissance De La Sardine (*Sardina Pilchardus* Walb, 1792) De La Région Centre Et Est De La Méditerranée Marocaine A L'aide De L'étude Des Otolithes

By Omar Kada, Souad Abdellaoui, Mohamed Najih & Driss Nachite

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Résumé - L'étude de la croissance de la sardine *Sardina pilchardus* qui peuple les côtes méditerranéennes marocaines, basée sur la lecture directe des otolithes extradites à partir de l'oreille interne, a permis l'estimation de l'âge de 1015 spécimens. Ces derniers ont été échantillonnés des débarquements réalisés dans les principaux ports de la Méditerranée marocaine durant les années 2002 à 2004. Les paramètres de croissance, basés sur l'équation de Von Bertalanffy ainsi que ceux relatifs à la relation taille-poids sont : L_∞ = 21,3 cm ; k = 0,56 ; t_o= –0,67 ; W_∞= 76,2 ; a= 0,0066 et b= 3,0582.

GJSFR-C Classification : FOR Code: 069999

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I. Introduction

La pêche sardinière exercée en Méditerranée marocaine par des senneurs, ciblant particulièrement la sardine « *Sardina pilchardus* », est une composante importante de la pêche côtière, et contribue significativement dans l'économie de pêche de la région Nord du Maroc, vue son rendement en termes de captures et le chiffre d'affaire qu'elle génère.

En effet, les débarquements de la sardine représentent environ 70% de l'ensemble des captures des petits pélagiques (KADA et al. 2003) réalisées par la flotte de pêche sardinière. Toutefois, et depuis l'année 1970, une pression de pêche de plus en plus élevée a été enregistrée et qui s'exerce en particulier sur la sardine.

Pour mieux comprendre la situation réelle de l'exploitation de la sardine méditerranéenne et l'état dustock de l'espèce en question, les études d'évaluation de stocks, notamment par les méthodes indirectes, permettent de mieux diagnostiquer la situation d'exploitation et élaborer des éléments scientifiques nécessaires à la mise en place d'un plan de gestion. La réalisation de ces études repose sur la détermination de certains paramètres caractéristiques de la population de la sardine méditerranéenne, notamment les données sur l'âge ainsi que les paramètres de la croissance de ladite espèce.

Fondamentalement, l'étude de la croissance revient à décrire un changement moyen par unité de temps. La croissance d'une population ou d'un individu est souvent représentée par des modèles mathématiques. Putter, 1920 (*in* Sparre *et al.* 1996) a élaboré un modèle de croissance dans lequel on peut voir la base de la plupart des autres modèles y compris le modèle mathématique de croissance individuelle pour la longueur ou le poids élaboré par Von Bertalanffy (1938).

La présente étude s'intéresse à l'estimation de l'âge de la sardine de la Méditerranée marocaine à travers la lecture directe des otolithes entiers, en vue d'estimer les paramètres biologiques relatifs à la croissance de cette espèce selon le modèle de Von Bertalanffy.

II. Matériel ET Méthode

L'échantillonnage biologique de la sardine provenant des captures commerciales des senneurs a été effectué au niveau des principaux ports situés au niveau de la région du centre et de l'Est de la Méditerranée marocaine : Al Hoceima, Nador et Cap de l'eau durant les années 2002, 2003 et 2004. Cet échantillonnage a couvre presque l'ensemble de gamme de taille de sardine et qui se situe entre 8 et 22,5 cm.

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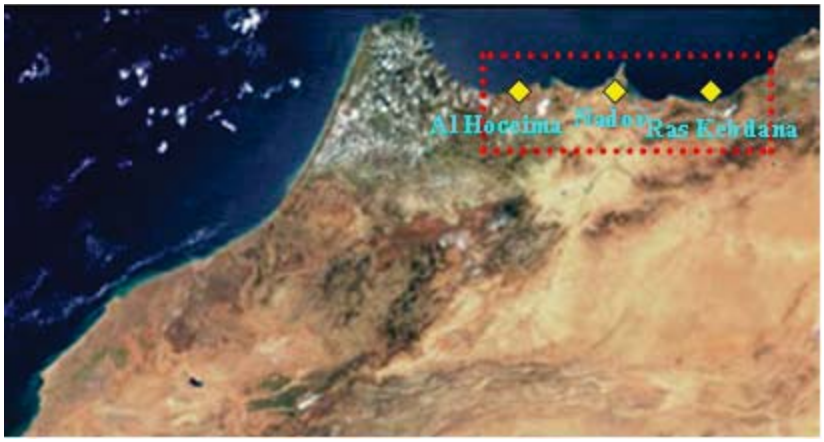

Figure 1 : Photo satellitaire montrant de la zone d'étude (Source : SIG-INRH, 2004)

Après leur extraction, les paires d'Otolithes sont placées dans des petits tubes répertoriés. Ensuite, ces pièces calcifiées, ont été montées en inclusion dans la résine polyester « EUKITT » sur des plaquettes noires et creusées d'alvéoles numérotées.

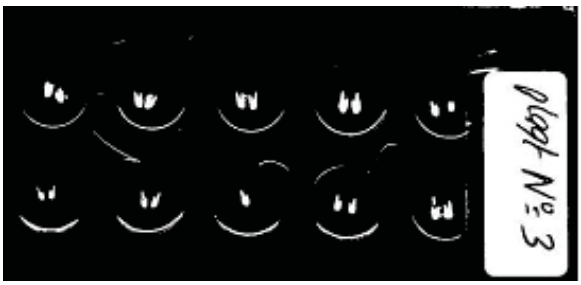

Photo 1 : Otolithes de sardine montées sur plaquette (Gr :x 1)

La lecture d'âge de la sardine a porté sur l'observation sous la loupe binoculaire (GR :X50) des stries de croissance des otolithes entiers montés sur des plaquettes noires. En vue de réaliser une bonne estimation d'âge et réduire les éventuelles erreurs d'analyse d'otolithe, trois lectures ont été effectuées par trois auteurs différents. L'âge adopté est celui qui présente une meilleure concordance entre les différentes lectures.

L'estimation des paramètres de croissance a été effectuée en se basant sur le modèle de Von Bertalanffy (1938). Ce modèle est appliqué pour la plupart des espèces de poissons (Sparre et al., 1996).

Après ajustement des données de longueur et d'âge observés, les trois paramètres de croissance (L_∞, k, t_0) ont été estimés, moyennant le logiciel « FISAT » en appliquant l'équation Von Bertalanffy. Pour cela, les données relatives aux tailles et les âges correspondant pour tous sexes inclus, ont été introduites dans ce logiciel de traitement de données.

L'expression du model de croissance est la suivante:

- Croissance linéaire: $L_t = L_\infty (1-\exp(-k^{(t-t_0)}))$
- Croissance pondérale: $W_t = W_\infty (1-\exp(-k^{(t-t_0)}))$

Lt: longueur du poisson à l'instant t ; L∞ longueur asymptotique qui serait atteinte par le poisson à l'âge théorique infini ; k : coefficient de croissance caractérisant la vitesse avec laquelle l'espèce croît vers sa taille asymptotique ; t_0 : âge théorique pour une longueur nulle ; Wt : poids du poisson à l'instant t ; W∞ : poids asymptotique qui serait atteinte par le poisson à l'âge théorique infini.

Les données utilisées pour déterminer la relation taille-poids de la sardine sont celles de la longueur totale (au millimètre près) et de poids (g). Pour la sardine, la formulation mathématique de l'équation de la croissance exprimant l'évolution des poids moyens en fonction du temps se fait par simple combinaison de la relation longueur-poids et de l'équation de croissance en taille. L'expression de la relation Taille-poids est de type exponentiel, s'écrit comme suit:

$$W_t = a.\ L^b$$

avec L : taille du poisson et P : poids qui lui correspond.

Cette relation qui lie la taille au poids a été calculée sous forme de coordonnées logarithmiques pour les deux sexes prisent ensemble et pour tous les individus composant les échantillons.

III. Résultat ET Discussion

Au total, 1143 paires d'otolithes ont été examinées dont 128 se sont révélées douteuses ou illisibles. L'estimation des âges a été effectuée sur presque l'ensemble des classes de taille qui sont de 1 cm d'intervalle et à chaque classe de taille sont réalisés plusieurs lecteurs d'otolithes appartenant à des individus différents.

a) Estimation des paramètres de croissance (L_∞, k et t_0)

Les résultats obtenus de la lecture des otolithes nous a permis d'établir une clé taille/âge. Les longueurs

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moyennes estimées par groupe d'âge ont été utilisées pour estimer les paramètres de la relation de croissance linéaire, et qui s'écrit comme suit:

$$L_t = 21{,}3\ (1\text{-exp}\ (-0{,}56^{(t+0.67)}))$$

L'analyse de la croissance effectuée sans distinction entre les sexes indique que la sardine a une longévité qui peut aller jusqu'à six ans (figure 2). De même, on note que cette espèce des clupéidés présente une croissance rapide durant sa phase juvénile notamment au cours des deux premières années. En effet, la longueur moyenne à 6 mois est de 10,6 cm et elle devient presque de 15,3 cm à 18 mois.

A l'âge de deux ans et demi, la sardine peut avoir une taille moyenne de l'ordre de 18 cm. Toutefois, et à partir de la troisième année, la croissance de la sardine en longueur devient de plus en plus lente et peut être estimée à quelques millimètres par an.

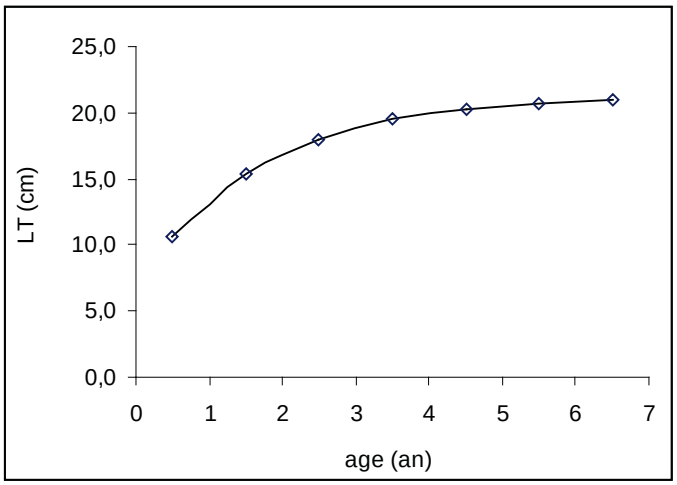

Figure 2 : Courbe théorique de la croissance linéaire de la sardine de la région Centre et est de la Méditerranée marocaine

Pour analyser les caractéristiques de la croissance de « *Sardina pilchardus* » des côtes méditerranéennes marocaines et faire des comparaisons avec les autres populations de cette espèce qu'abrite les autres régions de la Méditerranée et aussi celle de l'Atlantique marocain, nous avons élaboré le tableau 3, dans lequel sont exposées les valeurs des paramètres de l'équation de Von Bertalanffy, estimées pour la sardine de ces régions.

Au niveau de notre zone d'étude (côtes méditerranéennes marocaines), les paramètres de croissance (L_∞, k et t_0), qui caractérisent la sardine de cette région sont comparables à ceux observés pour la sardine du secteur Nord-Ouest de la Méditerranée.

Tableau 1 : Paramètres de l'équation de Von Bertalanffy relatifs à la croissance de la sardine dans différentes régions de la Méditerranée et Atlantique marocains

Zone géographique	L_∞ (cm)	k	t_o	Référence
W. Méditerranée (Iles Baléares)	21,2	0,39	-	GFCM, 1981
W. Méditerranée (Alicante)	22	0,29	-	Larrañeta, M.G., 1975.
W. Méditerranée (Mer d'Alboran)	20,69	0,69	-0,64	Alemany et al., 1993
W. Méditerranée (Catalan)	21,20	0,31	-	Pertierra et al., 1989
Atlantique marocaine	21,6	0,88	-0,129	Delgado et al., 1981.
Méditerranée marocaine	21,3	0,56	-0,67	Présente étude

b) Estimation des paramètres de la relation taille/poids (a et b)

La relation entre la taille de la sardine à sa masse corporelle est établie à partir de données de mensuration de la taille et du poids de 1197 individus, sans faire de distinction entre les sexes.

L'équation de cette relation taille/poids, se présente comme suit :

$$P = 0.0066.\ L_t^{\ 3{,}0582} \qquad (R^2 = 0{,}95)$$

La combinaison de l'équation de croissance linéaire de Von Bertalanffy avec la relation taille- masse, permet d'établir l'équation de croissance pondérale qui s'écrit :

$$W_t = 76.2*(1\text{-exp}\ (-0.56^{(t+0.67)}))$$

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IV. Conclusion

Cette étude a permis de caractériser la croissance chez la sardine méditerranéenne marocaine. Les paramètres relatifs à la croissance de la sardine en Méditerranée marocaine ont été estimés à partir de la lecture directe des otolithes entiers. Pour le deux sexe inclus, la sardine présente une allométrie de croissance, c'est à dire que la croissance en poids et en taille ne sont pas toujours proportionnelles avec l'âge. De même, la relation entre la taille de la sardine et son poids est légèrement majorante, qui tend vers une isométrie de croissance (en grandissant, les sardines grossissent un peu plus qu'elles ne s'allongent).

Les paramètres de croissance de l'équation de Von Bertalanffy qui caractérisent la sardine de la région de la Méditerranée marocaine ainsi que ceux relatifs à la relation taille-poids sont respectivement, L_∞ = 21,3 cm ; W_∞= 76,2 ; k = 0,56 ; t_0= -0.67 ; a= 0,0066 et b= 3,0582.

A la lumière des résultats obtenus dans cette étude, le programme concernant l'étude dynamique de population et d'estimation indirecte du stock de la sardine peut être dorénavant menée sur la base des données biologiques spécifiques à la population cette espèce en Méditerranée marocaine et sans faire recours aux données biologiques obtenues dans d'autres régions de la mer d'Alboran.

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GJSFR-C Classification : FOR Code: 961499, 050399

TRICHODERMASPP. ISOLATED FROM SOILS FROM THE SOUTHERN STATE OF TOCANTINS FOR THE CONTROL OF SCLEROTINIA SCLEROTIORUM IN VITRO

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Trichoderma spp. Isolated from Soils from the Southern State of Tocantins for the Control of *Sclerotinia Sclerotiorum* in Vitro

Lillian France Borges Chagas [α], Aloisio Freitas Chagas Junior [σ], Henrique De Castro Guilhon [ρ], Evilanna Lima Arruda [ω], Gil Rodrigues Dos Santos [¥] & Luciane Oliveira Miller [§]

Abstract - The objective of this work was to select isolates of *Trichoderma* spp. with potential of antagonism against *Sclerotina sclerotiorum,* in vitro. We used ten isolates of *Trichoderma* spp. and an isolate of *S.sclerotioruma* long with "inhibition by Volatile Products" and "direct confrontation" techniques. The results of in vitro procedures, lead to the selection of the isolates JCOUFT-28, JCOUFT-37, JCOUFT-45, JCOUFT-63 and JCOUFT-85, which were *Trichoderma* spp. that showed better antagonistic activity on the isolated *S.sclerotiorum*.

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I. Introduction

The state of Tocantins is partially located on the cerrado (savanna), an area with great potential for agriculture, cattle raising and cultivation of cereal grains. However, there are several factors that can lead to low productivity of agricultural areas. Primarily, plant disease caused by soil borne plant pathogens result in severe yield loss.

Sclerotinia sclerotiorum is a soil borne plant pathogen that causes white mold, which key characteristics are white, fluffy my celial growth on the host plant eventually producing specialized survival structures known as sclerotia (Cardoso, 1990). The increasing severity of the fungus is a reason for concern because the sclerotia can easily contaminate non-infected areas and can survive for years in the soil; moreover there are no resistant varieties to this disease (Niderasementes, 2009).

Isolates of *Trichoderma* spp. are considered efficient against several plant-pathogenic fungi. The mode of action of *Trichoderma* spp. is one or an association of the following: Parasistism, antibiosis, competition. *Trichoderma* is of saprophytic nature and produces extracellular enzymes and antibiotics that increase its parasitic capability, competitiveness and efficiency in biological control (Harman et al., 2004; Samuels, 2006).

Several researches have been done to evaluate the antagonist capability of *Trichoderma* spp against S. sclerotiorum, in vitro (Delgado et al., 2007; Louzada et al., 2009). However, no biological control study has been developed with isolates from the south of Tocantins, against *Sclerotinia sclerotiorum,* also isolated from the same region.

The present study´s objectives were to select isolates of *Trichoderma* spp. from the South of To cantins with potential to control *Sclerotinia sclerotiorum.*

II. Materials and Methods

In order to select isolates of *Trichoderma* spp., soil samples were cultured as a possible *Trichoderma* spp. sources. Thirty-seven soil samples were collected from the experimental areas of the Federal University of Tocantins-Gurupi campus, localized at 11°43'Se49°04'W with 300 m altitude and in valley areas of Lagoa da Confusão– TO (TABLE 1). The samples were taken at adepth of 0-10 cmin soil that had numerous crops and were cultivated by different methods. Afterwards, the samples were sent to the Plant Pathology Lab at the Federal University of Tocantins-Gurupi campus, where they were kept in a cold chamber until analysis.

Subsequently, five fragments of approximately 2 mm in diameter were placed in each petri dish, using the technique of direct isolation of fungi, with 3 repetitions for each sample, in the PDA (Potato, dextrose, agar) culture medium, and incubated at a temperature of 28 °C and a photoperiod of 12 hours. The period for colony growth of *Trichoderma* spp., was determined according to Watts et al. 1988. After 5 days, the petridishes with colony growth similar to *Trichoderma* spp. were selected, resulting in a total of 19 isolates found in the soil samples. In order to confirm the genus, the colonies were transferred to petri dishes that had PDA culture medium and after seven days of incubation at a temperature of 28 °C and a photoperiod of 12 hours they were identified based on morphology with the help of a microscope (Barnett & Hunter, 1998). All the isolates were maintained in the refrigerators, cultivated periodically on PDA and conserved on mineral oil for preservation of the isolates of *Trichoderma* spp. obtained.

The plant pathogen, S. sclerotiorum, was isolated from beans (*Phaseolus vulgaris* L.), that had high incidence of the disease and was cultivated in the experimental area of the Federal University of Tocantins-Gurupi Campus, and it was preserved in the same

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manner of the *Trichoderma* spp. The botanical material was collected and processed in 24 hours and it was previously abundantly washed in running water and neutral detergent to remove the epiphytic excess. Later, inside an aseptic chamber, the material was immersed in 70% alcohol for 1 minute and 3 % hypochlorite for 4 minutes and again in 70% alcohol for 30 seconds to remove the excess of hypochlorite. The same material was then washed in sterile distilled water of which a sample of 50 μL was taken for control. After the sterilization, small fragments measuring approximately 8 x 8 mm were place on PDA culture medium that had terramicina (100 μL) to inhibit bacterial growth during the fungus isolation. According to Monteiro et al. (2012) the agar substrate presents better carpogenic germination of sclerotia of *S. sclerotiorum*. The petri dishes containing the fragments were incubated at 28 °C.

Conidia counting of *Trichoderma* spp. of the 19 isolates previously obtained were done to compare the production of conidia to the control isolate, *T. harzianum* of the commercial product Trichoplus – JCO Fertilizantes, produced in Barreiras- Bahia.

Discs of 5 mm in diameter with mycelia growth of these isolates on PDA for 7 days were transferred to petri dishes (90 mm of diameter) also with the PDA culture medium. They were incubated in growth rooms acclimatized to 28°C ± 2°C, with a photoperiod of 12 hours. In order to evaluate the conidial production, 10 ml of distilled water was added to the petri dishes containing the colonies grown for 5 days. Using a Drigalski spatula, the medium containing the my celial and conidial growth, was lightly scraped off and agitated so that the spores loosen. The resulting suspension was filtered in double gauze and the concentration of the conidia was determined with the help of a Neubauer chamber. For both tests, complete randomized blocks were used with three repetitions per isolate.

With the intention of verifying if the isolates produced volatile metabolites a inhibition test for volatile products was done based o Bharat et al. (1980), where the lids were placed on top of each other after pouring PDA culture medium in each of them. The antagonist was positioned on the lower part of the petri dish while the pathogen was on the upper part. The antagonist was inoculated on the first, third and sixth day of growth of the pathogen. The lids were closed, laterally sealed by a flexible plastic and incubated at room temperature for seven daysunder continuous florescent light. The evaluation method consisted in measuring the radius of mycelia development to obtain the area of the colony.

In order to verify interaction between the pathogen and antagonist, the 19 isolates of *Trichoderma* spp. previously used were tested and evaluated with the methodology of direct confrontation (Bell et al., 1982; Ethur et al., 2005). Confirming the antagonisms of the *Trichoderma* spp. against the *S. sclerotiorum* was done using direct opposition method described by Dennis & Webster (1971). Initially, isolates were cultured on PDA medium. Petri dishes containing 20 ml of PDA medium each, received 2 discs of mycelia/agar of 9mm in diameter, which were placed on opposite ends and 1 cm apart from the side of the dish. One of the discs was a pathogen and the other one was the possible bio control agent.

As a control, petri dishes were cultured only with the pathogens. The colonies were placed in a BOD Incubator at a temperature of 28 °C ± 2°C and a 12 hour photoperiod. After seven days my celial growth of the colonies were measured with the help of a caliper, starting at the culture disc. The evaluation was done following the criteria proposed by Bell et al. (1982) with grading scales varying from 1 to 5. In this scale when the antagonist grows all over the dish (87, 5 to 100%) it is considered 1 and 6 when it does not present satisfactory growth (under 33, 2%). The isolate was considered efficient as an antagonist when the grade was lower or equal to 3. All the treatments were conducted with 3 repetitions and analyzed using a completely randomized factorial design.

The results obtained were examined using analysis of variance (ANOVA) and partitions performed by the Scott-Knott test at 5 % probability.

Table 1 : Origin of the isolates of *Trichoderma* spp. obtained from soil samples

Identification of Isolate	Origin	Crop
JCO-UFT 02	UFT campus - Gurupi-TO	Sorghum (*Sorghum bicolor*)
JCO-UFT 03	UFT campus - Gurupi-TO	Rice (*Oryza sativa*)
JCO-UFT 06	UFT campus - Gurupi-TO	Barbados Nut(*Jatrophacurcas*)
JCO-UFT 09	UFT campus - Gurupi-TO	Corn (*Zeamays*)
JCO-UFT 12	UFT campus - Gurupi-TO	Sugar-cane (*Saccharumofficinarum*)
JCO-UFT 14	UFT campus - Gurupi-TO	Capim Napier (*Pennisetumpurpureum*)
JCO-UFT 15	UFT campus - Gurupi-TO	Watermelon (*Citrulluslanatus*)
JCO-UFT 18	UFT campus - Gurupi-TO	Castor oil plant (*Ricinuscommunis*)
JCO-UFT 19	UFT campus - Gurupi-TO	Lemon grass (*Cymbopogoncitratus*)
JCO-UFT 22	UFT campus - Gurupi-TO	Vegetação de Mata
JCO-UFT 23	UFT campus - Gurupi-TO	Pinaple (*Ananascomosus*)
JCO-UFT 25	UFT campus - Gurupi-TO	Banana (Musa spp.)

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JCO-UFT 28	UFT campus - Gurupi-TO	corn(*Zeasmays*)
JCO-UFT 32	UFT campus - Gurupi-TO	corn(*Zeamays*)
JCO-UFT 34	UFT campus - Gurupi-TO	Pumpkin (*Cucúrbita moschata*)
JCO-UFT 35	UFT campus - Gurupi-TO	Cerrado vegetation
JCO-UFT 37	UFT campus - Gurupi-TO	Degradedpasture
JCO-UFT 41	UFT campus - Gurupi-TO	Balm (*Melissa officinalis*)
JCO-UFT 45	Lagoa da Confusão-TO	Sunnhemp(*CrotaláriaJuncea*)PD*
JCO-UFT 48	Lagoa da Confusão-TO	Sunnhemp(*CrotaláriaJuncea*) PC*
JCO-UFT 56	Lagoa da Confusão-TO	Crotalaria (*CrotaláriaSpectabilis*) PC*
JCO-UFT 57	Lagoa da Confusão-TO	Velvetbean(*Mucunaaterrima*) PD*
JCO-UFT 63	Lagoa da Confusão-TO	Jack bean (*Canavaliaensiformis*) PD*
JCO-UFT 67	Lagoa da Confusão-TO	Jack bean(*Canavaliaensiformis*) PC*
JCO-UFT 70	Lagoa da Confusão-TO	Hyacinth bean (*Lablabpurpureus*) PD*
JCO-UFT 74	Lagoa da Confusão-TO	Hyacinth bean (*Lablabpurpureus*) PC*
JCO-UFT 76	Lagoa da Confusão-TO	Pigeon Pea(*Cajanuscajan*) PD*
JCO-UFT 78	Lagoa da Confusão-TO	Pigeon Pea (*Cajanuscajan*) PC*
JCO-UFT 85	Lagoa da Confusão-TO	Calopogônio(*Calopogoniummucunoides*) PC*
JCO-UFT 87	Lagoa da Confusão-TO	Radish(*Raphanussativus*) PD*
JCO-UFT 92	Lagoa da Confusão-TO	Radish(*Raphanussativus*) PC*
JCO-UFT 95	Lagoa da Confusão-TO	No cultivationof legumes
JCO-UFT 96	Lagoa da Confusão-TO	No cultivationof legumes
JCO-UFT 99	Lagoa da Confusão-TO	Cowpea (*Vignaunguiculata*) PD*
JCO-UFT 102	Lagoa da Confusão-TO	Cowpea (*Vignaunguiculata*)PC*
JCO-UFT 110	Lagoa da Confusão-TO	Sorghum(*Sorghum bicolor*) PC*
JCO-UFT 111	Lagoa da Confusão-TO	Natural Meadowvegetation

* PD- Plantio direto; PC- Plantio convencional.

III. Results and Discussion

The concentration of conidia of *Trichoderma* spp. was determinedand compared to the control isolate. Only the isolates JCO-UFT 28 and JCO-UFT 35 presented a lower number of conidia when compared to the control (Trichoplus). The other isolates had a superior number of conidia than the control, as shown on table 2.

Table 2 : Results of conidia counting of *Trichoderma* spp. collected and isolated from the South of state of Tocantinsand the control sample of the commercial formulation Trichoplus JCO from Barreiras- BA

Identification	Numberofconidia
JCO-UFT 22	$1,7.\ 10^6$
JCO-UFT 28	$2,5.\ 10^5$
JCO-UFT 32	$1,5.\ 10^6$
JCO-UFT 35	$1,6.\ 10^5$
JCO-UFT 37	$6,3.\ 10^6$
JCO-UFT 41	$7,0.\ 10^6$
JCO-UFT 45	$8,2.\ 10^5$
JCO-UFT 57	$1,1.\ 10^6$
JCO-UFT 63	$5,5.\ 10^5$
JCO-UFT 67	$1,4.\ 10^6$
JCO-UFT 76	$5,5.\ 10^5$
JCO-UFT 78	$1,1.\ 10^6$
JCO-UFT 85	$5,3.\ 10^6$
JCO-UFT 87	$2,7.\ 10^6$
JCO-UFT 92	$6,4.\ 10^5$
JCO-UFT 95	$1,3.\ 10^6$
JCO-UFT 99	$2,7.\ 10^6$
JCO-UFT 102	$1,9.\ 10^6$
JCO-UFT 110	$1,6.\ 10^6$
CONTROL	$3,9.10^5$

The test for inhibition of volatiles products the isolates JCO-UFT 63, JCO-UFT 78, JCO-UFT 92 and JCO-UFT 102 produced some type of volatile compound, showing a percentage of growth reduction (GR) of the mycelia of *S. sclerotiorum*. When inoculated 3 days after the pathogen (figure 1), the antagonist was

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able to reduce up to 60 % of the pathogen growth when compared to the control. These results are in agreement with Mace do et al. (2007) findings that show the inhibition of S. *rolfssimycelial* growth by 12 isolates of *Trichoderma* spp. tested.

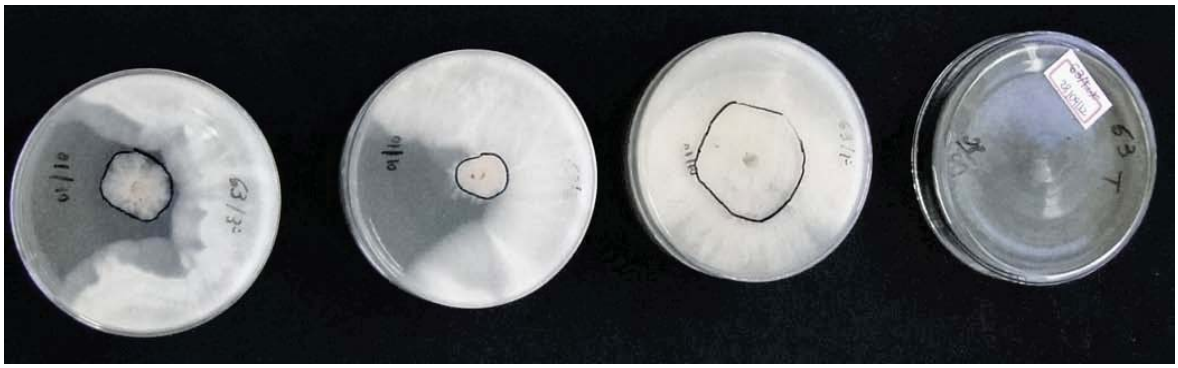

Figure 1 : Inhibition of the pathogen *(S. sclerotiorum)* by *Trichoderma* JCO-UFT 63, inoculated 3 days after the pathogen, part of the volatile production test

As for the direct opposition test, of the 19 isolates identified belonging to the *Trichoderma* genus, 16 isolates inhibited the mycelial growth of *S. sclerotiorum* with evaluations smaller than 3 as shown on table 3.The isolates JCO-UFT 28, JCO-UFT 37, JCO-UFT 45, JCO-UFT 63 and JCO-UFT 85 were significantly efficient as antagonists when compared to the other isolates. They presented grades equal to or smaller than 2, where they took over more than 2/3 of the petri dish. Only the isolates JCO-UFT 22, JCO-UFT 41, JCO-UFT 95 and JCO-UFT 99 were considered inefficient ranking 3 or higher, in other words, occupying less than half of the petri dish.

The direct opposition between the pathogen and the antagonist showed the isolates of *Trichoderma* utilized different methods of antagonism besides the production of volatile metabolites. According to Melo (1998) the mode of action of *Trichoderma* is a result of an association of mechanisms such as parasitism, antibiosis and competition. A dark colored halo was observed along the contact line between the colonies of the antagonist and the plant pathogen, as previously described by Bell et al. (1982) and Durman et al. (1999). The sclerotia formed in the petri dishes were colonized by the isolates of *Trichoderma* spp., and then they fragmented and lost their rigid consistency. Melo (1991) stated that the fungus *Trichoderma* has been found growing on sclerotia, structures that are known to be very resistant to parasitism.

Table 3 : Antagonistic activity, in vitro, of the isolates of *Trichoderma* spp., to the isolates of *S. Sclerotinia*[1]

Identification	Distance (cm)	Grade	Medium %
JCO-UFT 22	2,69	3,5	40 c
JCO-UFT 28	1,87	2	63 a
JCO-UFT 32	1,78	2,5	55 b
JCO-UFT 35	2,19	2,5	57 b
JCO-UFT 37	1,58	1,5	68 a
JCO-UFT 41	2,33	3,5	48 c
JCO-UFT 45	1,93	2	63 a
JCO-UFT 57	1,90	2,5	58 b
JCO-UFT 63	1,66	2	66 a
JCO-UFT 67	2,27	2,5	54 b
JCO-UFT 76	2,11	2,5	55 b
JCO-UFT 78	2,10	2,5	57 b
JCO-UFT 85	1,83	2	65 a
JCO-UFT 87	2,13	2,5	59 b
JCO-UFT 92	1,85	2,5	58 b
JCO-UFT 95	3,30	4	37 d
JCO-UFT 99	3,56	5	28 d
JCO-UFT 102	2,10	2,5	56 b
JCO-UFT 110	2,07	2,5	58 b
CONTROLE	2,18	2,5	55 b

[1]The colonies developed from 2 discs of the 2 fungi, place in opposite direction, at the borders of the petri dish containing PDA medium. Medium followed by the same letter are not different in the Scott-knott 5% test.

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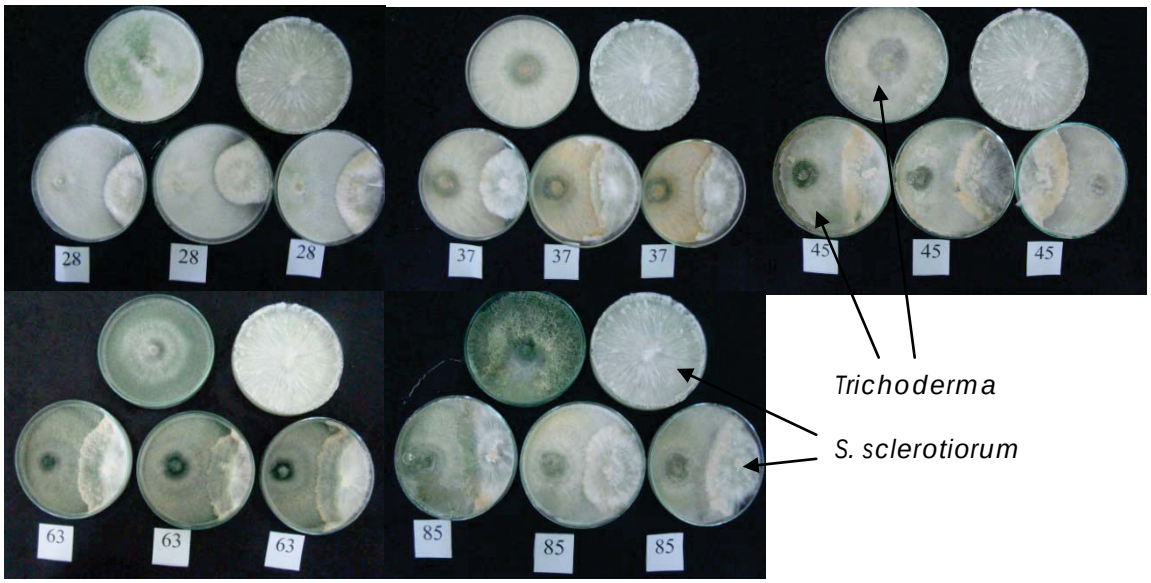

Figure 2 : Inhibition of the pathogen *(S. sclerotiorum)* by isolates of *Trichoderma spp.*, using direct opposition

IV. Conclusions

- The isolates JCO-UFT 63, JCO-UFT 78, JCO-UFT 92 and JCO-UFT 102 caused the most inhibition of the *S. sclerotiorum* growth when the antagonist was inoculated 3 days after the pathogen.
- The isolates JCO-UFT 28, JCO-UFT 37, JCO-UFT 45, JCO-UFT 63 and JCO-UFT 85 were efficient antagonist, inhibiting more than 60% of growth of the pathogen.

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3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
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- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

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The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
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Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
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What to keep away from

- Resources and methods are not a set of information.
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- Leave out information that is immaterial to a third party.

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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

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- Present a background, such as by describing the question that was addressed by creation an exacting study.
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Approach

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Approach:

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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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