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Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-8, August 2018, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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

Environmental Research:

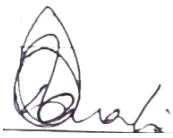
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment,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

Agriculture Research:

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

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

Mukesh Arora
(Editor-in Chief)



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Soil Science	Plant Science
Animal Science	Agricultural Economics
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Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
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Purification and properties of polygalacturonase associated with the infection process of *Colletotrichum truncatum* CP2 in chilli

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Abstract*—In this study, polygalacturonase enzyme produced by Colletotrichum truncatum CP2 was partially purified by aqueous two-phase system and the properties of this enzyme was characterized. The highest yield (57.4%) and purification fold (5.1) was obtained using 22% PEG 6,000/15% sodium citrate comprising crude load of 16% (w/w) at pH 7.0 with addition of 1.0% (w/w) sodium chloride. The partially purified PG remained active over a wide range of pH (2.5-6.0) and the optimum activity was obtained at pH 5.0. Incubation of the partially purified PG at 40 and 50 °C for 30 min caused the activity of PG to decrease up to 20% and 40%, respectively. However, no significant changes in the activity when the enzymes were incubated up to 4 h at 40 and 50 °C. The results from this study suggested that ATPS comprising of PEG and sodium citrate could be potentially used as an alternative method for purification of PG.*

Keywords*— *Chilli, Colletotrichum truncatum, polygalacturonase, aqueous two-phase system.

I. INTRODUCTION

The plant cell wall is a major barrier to the establishment of fungal infection on a host. Most plant-pathogenic fungi produce a number of cell wall-degrading enzymes when grown in liquid culture containing pectin. One of these enzymes, PG has been implicated routinely in facilitating the invasion and colonization of host tissue during pathogenesis of fungal pathogens (Choi *et al.*, 2013). Highly purified PG from many fungal pathogens has been proved by several researchers to have the ability to cause cell maceration and kill tissues in a similar way to that seen in soft-rot diseases (Protsenko *et al.*, 2010; Herbert *et al*., 2004; Oeser *et al*., 2002).

Many organisms produces polygalacturonases, for example, bacteria, parasites and yeast (Jurick *et al.*, 2009; Latif & Sohail, 2012). Microbial PG from different microbial sources shows wide variety in their physicochemical and biological properties. Most of the PG was found optimum at pH range of 3.5 to 5.5 and temperature between 30 to 50 °C. Molecular mass of the PG also varies from 25 kDa to 85 kDa.

Microbial PG has to be purified for the complete understanding of its properties. Numerous purification strategies have been reported for PG all with varying degree of success. The purification methods commonly employed include ammonium sulphate precipitation, ion-exchange chromatography, Sephadex G-25 gel filtration, ultrafiltration, gel permeation chromatography and ethanol precipitation (Deshmukh *et al*., 2012; Jurick *et al*., 2009; Thakur *et al.*, 2010). Each purification method has its own drawback associated with low yield and purity, cost and the requirement for a skilled operator (Shaligram & Singhal, 2010).

The ATPS has been proposed as an ideal and versatile strategy for the extraction and purification of biomolecules because of its high productivity, environmental-friendly, simplicity, short processing time, cost effectiveness and ease of scaling-up (Naganagouda & Mulimani, 2008; Raja *et al*., 2012). ATPS which consist of PEG/ salt system has been generally employed for the bioseparation of proteins due to its availability at low cost and wide range of hydrophobic differences between the two phase systems which allow enhancement of the partition selectivity of the target protein (Mehrnoush *et al*., 2011). ATPS has been applied in the extraction and purification of various compounds such as enzymes, biopharmaceuticals ad natural products (Srinivas & Raghavarao, 2000).

Selection of ATPS as a purification method is usually dependent on the types of biomolecules and economic considerations. Since the polymer/polymer system is very costly, the aqueous two phase polymer/salt systems are often used compared to the polymer/polymer systems (Abbasiliasi *et al.*, 2014). Moreover, polymer/salt systems have significant differences in density,

greater selectivity, lower viscosity, lower cost and the larger relative size of the drops (Nadar *et al*., 2017). Phosphates and sulfates are commonly used salts in polymer/salt ATPS. But the use of these salts has contributes to environmental problems, especially high concentrations of phosphate and sulfates in the effluent streams. Currently, the use of citrate salts as one of the ATPS components with PEG is preferred since citrate salts are biodegradable and non-toxic (Glyk *et al.*, 2015).

In view of the fact that ATPS is an ideal purification technique for biomolecules such as enzymes, this study evaluated the partitioning efficiency of a PG produced by *C. truncatum* CP2 using ATPS which comprised of PEG and sodium citrate. Since the partitioning mechanism in ATPS is still unknown, the effects of various parameters on the partitioning of the PG, such as the molecular weight, salt concentration, pH, crude load and addition of sodium chloride (NaCl) were investigated. The physico-chemical properties of partially purified PG were also characterized.

II. MATERIAL AND METHOD

2.1 Chemicals

Different molecular weight (MW) of PEG, ranging from 4,000 (g/mol) to 10,000 (g/mol), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium citrate was supplied from SAFC (St. Louis, MO, USA). The protein assay kit and albumin standard were obtained by Bio-Rad, USA and Thermo Scientific Pierce, respectively. All chemicals and reagents used were analytical grade.

2.2 Microorganism and polygalacturonase production

The polygalacturonase producing strain used in this study was *C. truncatum* CP2 which was isolated from lesions of chilli anthracnose**.** Polygalacturonase enzyme was produced in a shaking flask in the basal medium containing pectin from citrus as a carbon source. The fermentation broth was filtered through Whatman filter paper and centrifuged prior to purification by ATPS. The filtrate was used an enzyme source.

2.3 Construction of ATPS

The aqueous two phase system was prepared from stock solution of 50% (w/w) PEG with different MW (PEG 4,000, 6,000, 8,000 and 10,000 g/mol) and 40% (w/w) of sodium citrate stock solutions. The ATPS system was prepared in 15 mL centrifuge tubes. The amount of PEG solution, citrate solution and distilled water were weighed appropriately and mixed with crude enzymes 16% (w/w) to form a 10 g system. The solution was stirred thoroughly using a vortex mixer and then centrifuged at 2,860 × g for 10 min to achieve the phase separations. With the use of pipette, the upper phase was removed and the lower phase was then collected. The volumes of both phases were measured and the PG activity and total protein concentration of each phase were then analysed.

2.4 Polygalacturonase activity assay

Polygalacturonase (PG) activity, the method of Nelson and Somogyi (Nelson, 1944; Somogyi, 1952) was used to measure the release of reducing groups from polygalacturonic acid. The reaction mixture contained 1.8 mL of 1% PGA in 50 mM sodium acetate buffer (pH 4.8) and 0.2 mL of crude enzyme. For the control, the reaction mixture contained the same components except the enzyme was boiled for 5 min. The reaction mixture was then incubated at 40 °C for 30 min followed by addition of 3 mL of 3,5-dinitrosalicyclic (DNS) reagent. The reaction mixture was then heated at 100 °C for 15 min. Then, it was allowed to cool down at room temperature before addition of 1 mL Rochelle salt. The absorbance was measured at 545 nm using a spectrophotometer. The formation of reducing sugars was calculated using D-galacturonic acid as a standard. The amount of enzyme releasing 1 µmol of galacturonic acid per min at pH 4.8 and 40 °C was considered as one enzyme unit.

2.5 Protein concentration determination

The amount of protein concentration was determined using a Bicinchonic Acid (BCA) assay kit with the used of Bovine serum albumin (BSA) as a standard. A total of 50 µL of the sample was mixed with 1 mL of working reagent and then incubated at room temperature for 2 h. The absorbance was measured at 562 nm using a UV spectrophotometer (GENESYS 20, Thermo Scientific, UK).

2.6 Optimization of ATPS parameters

Standard experimental parameters in the aqueous two-phase extraction including MW of PEG, pH, concentration of PEG and sodium citrate in each phase, the addition of sodium chloride and the amount of loaded crude enzyme (C_L) were optimized

for PG recovery. Optimization was done (in triplicates) based on the experimental default conditions set at: total mass of aqueous phase = 10 g, crude load, 16% (w/w), PEG concentration = 50% (w/w) and pH = 7.0.

2.7 Determination of partition coefficient (K), yield of PG (%) and purification factor (P_F)

The partition coefficient of PG was determined by dividing the PG activity in the top phase with the PG activity in the bottom phase as shown below:

$$K = \frac{AT}{AB} \quad [1]$$

Where:

A_T and A_B is the PG activity in the upper and bottom phases, respectively.

In order to evaluate the purification process, the PG specific activity **(SA)**, the purification factor **(P_F)** and the enzyme yield recovered in the upper **(Y_T)** phases were also calculated, according to the given equations:

$$SA\left(\frac{U}{mg}\right) = \frac{PG\ activity\ (U/mL)}{[protein\ ](mg/mL)} \quad [2]$$

$$PF = \frac{SA\ of\ phase\ sample}{SA\ of\ crude\ feedstock} \quad [3]$$

$$YT = \frac{100}{1+[\frac{1}{VR}\times KE]} \quad [4]$$

$$VT = \frac{VT}{VB} \quad [5]$$

Where:

VT is the volume ratio, and V_T and V_B are the volumes of upper and bottom phases, respectively.

2.8 Characterization of partially purified polygalacturonase

The partially purified enzyme from *C. truncatum* CP2 was characterized and its different properties were examined. The characteristic studies include: substrate specificity and effect of temperature and pH on enzyme stability. The experimental procedures are performed as below.

2.8.1 Substrate specificity

Substrate specificity of the partially purified enzyme was studied by using different substrates in the reaction mixture for enzyme assay. The enzyme assays were performed under standard conditions with a fixed substrate concentration of 0.5% (w/v). The various substrates used were citrus pectin, apple pectin, xylan and CM-cellulose (Kant *et al*., 2013).

2.8.2 Effect of pH on enzyme activity and enzyme stability

The optimal pH for enzyme activity was determined by incubating the reaction mixture at different pH values using different buffers (50 mM sodium acetate for pH 3-4.5, 50 mM sodium citrate for pH 5-5.5 and 50 mM sodium phosphate buffer for pH 6-9). The PG activity was measured under standard assay conditions. For pH stability determination, the enzyme was preincubated at different pH values ranged from pH 2.5-7.5 at 4 °C for 2 h. The residual activity for PG enzyme was assayed with PGA as a substrate soon after incubation.

2.8.3 Effect of temperature on enzyme activity and stability

To determine the effect of temperature on the enzyme activity, the reaction mixture was incubated at different temperatures (30 to 90 °C) for 30 min and the activity was measured. For determination of thermal stability, the enzyme was incubated for variable durations (30 min to 4 hours) at fixed temperatures (40 to 50 °C). The residual activity was assayed soon after the incubation period.

III. RESULTS AND DISCUSSION

3.1 Effect of PEG molecular weight on partitioning of PG

The properties of polymer including concentration and its molecular weight are probably the most vital factors affecting the separation of biomolecules in different phases (Yang *et al*., 2013). Therefore, the effect of different molecular weight of PEG

(4,000-10,000 g/mol) on partitioning of PG was studied. The results show that the partition coefficient decreased as the PEG molecular weight increased (Table 1). This was due to a reduction in the volume to accommodate the target enzyme in the upper phase (PEG-rich phase) as PEG molecular weight increased. Volume exclusion usually occurs when high molecular weight of PEG is used due to the lack of molecular space for the enzyme (Benavides *et al.*, 2011; Grilo *et al.*, 2016). This will ultimately partitioned the PG enzyme in the bottom phase and consequently decreased the partition coefficient of the system.The highest purification factor was obtained with PEG 6,000 (2.10) indicating that there was an appropriate volume available to accommodate PG in the upper phase. PG yield was decreased when the PEG molecular weight increased which was most likely due to increase partitioning of PG to the lower phase. In addition, decreasing of purification factor of PG at high PEG molecular weight is due to the addition of PG to lower phase compared with other proteins (Mokhtarani *et al.*, 2008). Based on the results obtained, PEG 6,000 was selected as the most suitable molecular weight for further studies.

TABLE 1
INFLUENCE OF PEG MOLECULAR WEIGHT AND CONCENTRATIONS ON THE PARTITIONING BEHAVIOR OF PG

MW of PEG[a] (g/mol)	Partition coefficient (K)	Yield (%)	Purification factor
4,000	0.55^{A}	69.39^{A}	0.96^{A}
6,000	1.12^{B}	42.02^{B}	2.10^{B}
8,000	0.63^{C}	36.36^{C}	0.80^{C}
10,000	0.43^{C}	31.81^{C}	0.72^{C}

Note: [a] 22% (w/w) PEG + 16% (w/w) sodium citrate. Values within a column followed by different letters are significantly different at ($p < 0.05$).

3.2 Influence of salt concentration on the partitioning behavior of PG

The effect of different concentration of sodium citrate (12-16%, w/w) on the partition behaviour of PG from *C. truncatum* CP2 was investigated. Based on the results obtained, the purification factor and yield of PG was increased when the concentration of sodium citrate was increased from 12 to 15 % (w/w) (Fig. 1). The increased in purification factor and yield is due to the salting out effect of sodium citrate which caused increased PG partition in upper phase of the system. However, further increase in sodium citrate concentration up to 16% (w/w) has led to a decrease in the yield and purification factor. This could be due to the contaminating proteins being moved to the top phase which reduced the purity of the enzyme. These results are in agreement with the finding of Mehrnoush *et al*. (2011) who reported that increased of potassium phosphate concentration up to 20% (w/w) would decrease the purification factor and yield of pectinases from mango. Thus, the subsequent experiment was performed with 22% PEG 6,000/ 15% sodium citrate combination.

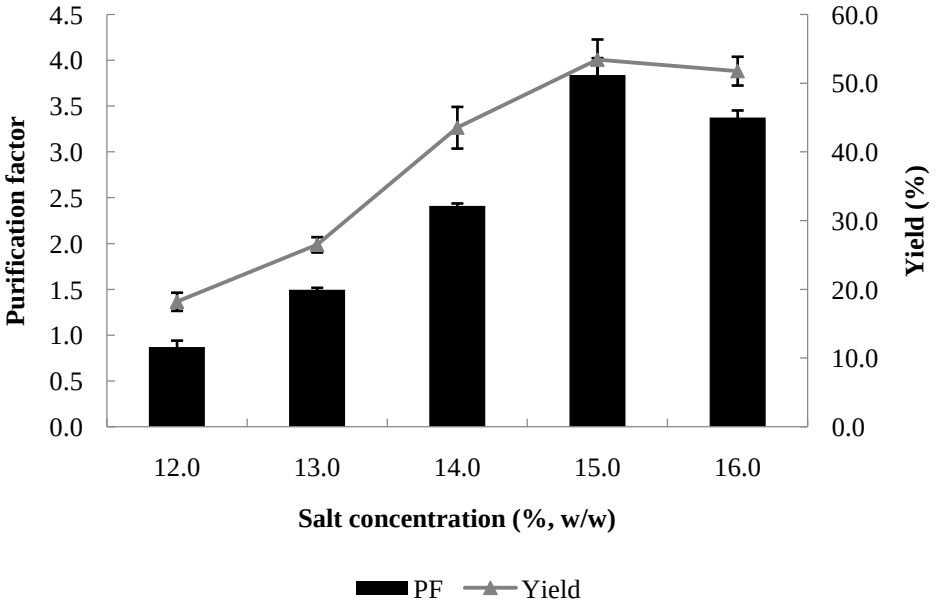

FIG. 1: INFLUENCE OF SALT CONCENTRATION ON THE PARTITIONING BEHAVIOUR OF PG. VALUES ARE MEANS OF 3 REPLICATES WITH ± SD.

3.3 Influence of pH on the partitioning behavior of PG

The effect of pH on partitioning of PG is important as the surface charge of protein is highly influenced by the pH of the system (Ooi *et al*., 2009). To achieve higher performance in partitioning of PG in ATPS, a suitable pH should be selected (Rahimpour & Baharvand, 2009). The partitioning behaviour of PG in ATPSs with different pHs are shown in Table 2. As shown in the table, the purification factor, partition yield and selectivity were increased with an increase of pH from 6.0 to 7.0. The purification factor (3.6), partition coefficient (0.91), yield (55%) and selectivity (1.84) were the highest when pH 7 was used. At this pH, most of the PG was partitioned to the upper phase. This was due to the effect of protein charge of PG (Benavides *et al*., 2011). The isoelectric point of PG is 6.1. At the isoelectric point of the protein, the sum of all the charges on the protein is zero. When the solution pH changes from acidic value to basic value, the protein become less positively or more negatively charge (Ratanapongleka, 2010). The charge of PG tends to be negative at pH 7 while PEG tends to be positive. This caused the attraction of PG to the positively charge PEG. However, a sudden reduction in the purification factor, partition coefficient, yield and selectivity were observed at pHs above 7. Higher pH was not favourable as the partition coefficient, yield and purification factor were decreased. This was due to the change of electrostatic interaction of contaminating proteins (Md Sidek *et al.*, 2016). The negatively charge contaminating proteins tends to be partitioned into the top phase at higher pH. Thus, pH 7 was selected as the optimum and used for the subsequent experiments.

TABLE 2
EFFECT OF PH ON THE PARTITIONING BEHAVIOUR OF PG. THE PH WAS VARIED FROM 6.0 TP 9.0. THE PURIFICATION FACTOR (PFT), PARTITION COEFFICIENT (K), YIELD (%) AND SELECTIVITY (S) OF PG WERE CALCULATED AND PLOTTED AT DIFFERENT PHS.

pH value[a]	Purification factor (P_{FT})	Partition coefficient (K)	Yield (%)	Selectivity (S)
6.0	1.0^{A}	0.03^{A}	10.9^{A}	0.01^{A}
6.5	2.1^{B}	0.34^{B}	45.2^{B}	1.50^{B}
7.0	3.6^{C}	0.91^{C}	55.0^{C}	1.84^{C}
7.5	1.4^{C}	0.41^{D}	50.1^{C}	0.20^{D}
8.0	1.0^{C}	0.39^{D}	44.5^{D}	0.16^{D}
8.5	1.1^{C}	0.34^{D}	42.5^{D}	0.14^{D}
9.0	0.9^{C}	0.33^{D}	43.3^{D}	0.11^{D}

Note: [a]22% (w/w) PEG + 15% (w/w) sodium citrate. Values within a column followed by different letters are significantly different at ($p < 0.05$).

3.4 Influence of crude load on the partitioning behavior of PG

The crude load has its own significance effect on the partitioning behaviour of PG. Fig. 2 shows the influence of crude load ranging from 10% (w/w) to 22% (w/w) on the partitioning behaviour of PG. The purification factor and yield were increased linearly with an increase of crude load from 10% (w/w) to 16% (w/w). However, the yield and purification factor start to decrease when crude stock loading higher than 16% (w/w) were used. Higher amount of crude extract may alter the properties of ATPS, which in turn affected the biomolecules partitioning (Asenjo & Andrews, 2012; Raja *et al*., 2012). The higher accumulation of PG at the Peg and salt interfaces was not favourable as it may cause the loss of PG activity. Hence, 16% (w/w) crude stock loading was chosen for further study.

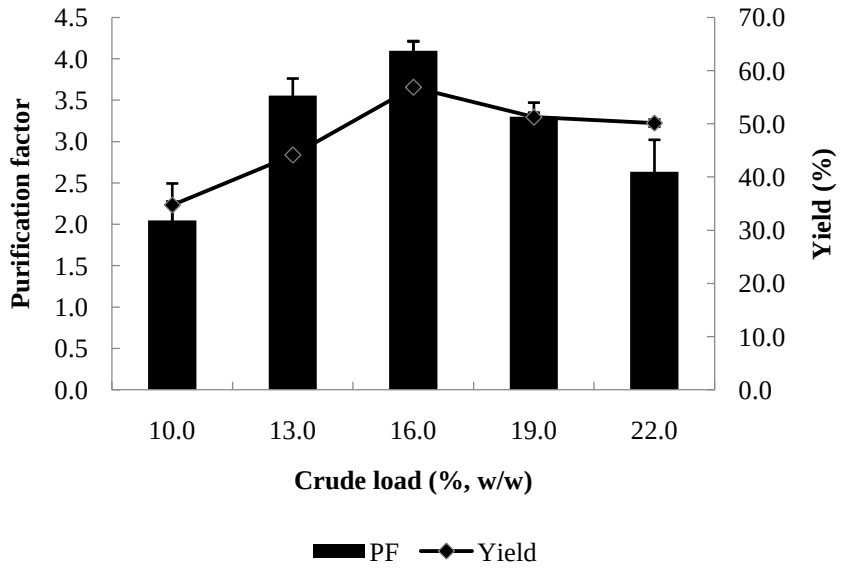

FIG. 2: INFLUENCE OF THE CRUDE LOAD ON THE PARTITIONING BEHAVIOUR OF PG. VALUES ARE MEANS OF 3 REPLICATES WITH ± SD.

3.5 Influence of NaCl on the partitioning behavior of PG

The effect of adding of NaCl, ranging from 0 to 3.0% (w/w) was studied for 22% PEG 6,000/15% sodium citrate with crude load 16% at pH 7.0 and the results is illustrated in Fig. 3. The addition of NaCl to the ATPS causes a hydrophobic difference in both phases increases thereby enhances the partitioning of hydrophobic proteins into the top phase. The addition of NaCl to the ATPS also improves the interaction between hydrophobic chain of PEG and the hydrophobic surface of PG. The optimum partitioning condition for PG was achieved with 1.0% (w/w) addition of NaCl. At this optimum point, PG exhibited the highest purification factor of 5.1, as well as the yield (57.4%). However, the yield and purification factor were decreased with an increase in NaCl concentration more than 1.0% (w/w). Ratanapongleka (2010) suggests that the addition of salt at high concentrations may lead to salt aggregation followed by protein precipitation because most of the water molecules were strongly attached to the salts. The interactions between proteins become stronger than between protein and water. Hence, the purification of PG in the subsequent experiments was carried out with addition 1.0% (w/w) of NaCl.

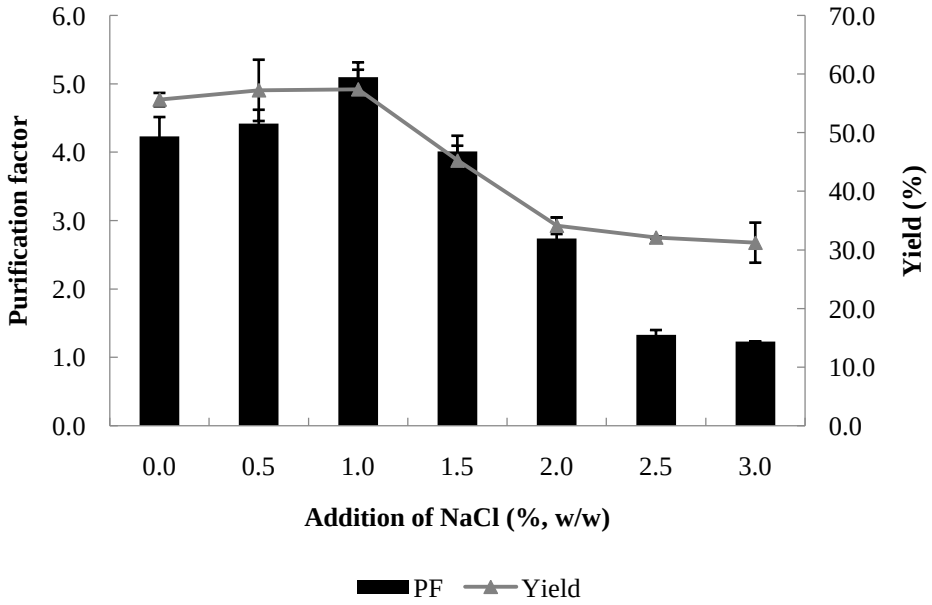

FIG. 3: INFLUENCE OF NACL ADDITION ON THE PARTITIONING BEHAVIOUR OF PG. VALUES ARE MEANS OF 3 REPLICATES WITH ± SD

3.6 Properties of partially purified PG

3.6.1 Substrate specificity

The specificity of the partially purified enzyme was evaluated with various substrates. The maximum PG specificity was observed when PGA was used as a substrate (Table 3). The enzyme also had a remarkable specificity with citrus pectin (81.2%) and apple pectin (64.9). Similar results were obtained by Martins *et al.* (2007) for PG from *Thermoascus aurantiacus*. Very low activity of enzyme was observed toward non-pectic polysaccharides (xylan and CMC). These results revealed that PG produced by *C. truncatum* CP2 had very high affinity and specificity toward PGA and pectins.

TABLE 3
SUBSTRATE SPECIFICITY OF PG FROM *C. TRUNCATUM* CP2 TOWARD DIFFERENT SUBSTRATES

Substrate	Relative activity (%)
PGA	100
Citrus pectin	71.2
Apple pectin	64.9
Xylan	10.1
CMC	8.4

3.6.2 Effect of pH on the activity and stability of PG

The influence of pH on the activity and stability of PG is shown in Fig. 4. Partially purified PG from *C. truncatum* CP2 was found to have optimum at pH 5.0. PG retained above 50% of its maximum activity in a broad pH range of 2.5-6.0. The pH optimum for activity of PG from *C. truncatum* CP2 is in good agreement with the optimum pH reported for PGs from *Aspergillus flavus*, *Trichoderma harzianum* and *Cylindrocarpon destructans* (Anand *et al*., 2017; Sathiyaraj *et al*., 2011). It is also very close to the pH optimum of *Rhizomucor pusilis* PG found by Siddiqui *et al*. (2012). Either increase or decrease in pH beyond the optimum value showed decline in enzyme activities. This PG was inactive at pH 8.0. Previous reports on biochemical properties shows that majority of fungal PGs are optimum at pH in acidic range (El-Batal *et al.*, 2013; Pathak *et al.*, 2000; Quiroga *et al.*, 2009). In the present study, it was observed that the maximum stability of PG enzyme was between pH 4.5 and 5.5. The results showed that the enzyme was very stable at pH 5.0 and retained 84.5% and 79.2% of its activity at pH 4.5 and 5.5, respectively. The enzyme lost about 80-90% of its activity at pHs 2.5 to 3.0 and pHs 7.0 to 9.0. This observation agreed with the results reported for PGs from *Trichoderma harzianum.* PG from *Mucor flavus* was found to be optimum at pH 4.0 to 6.0 but at pH 7.0 the stability decreased up to 80% (Saad *et al.*, 2007). Similar observation was reported by Gadre *et al*. (2003) in which the stability of PG from *Mucor flavus* decreased to 60% at pH 7.0.

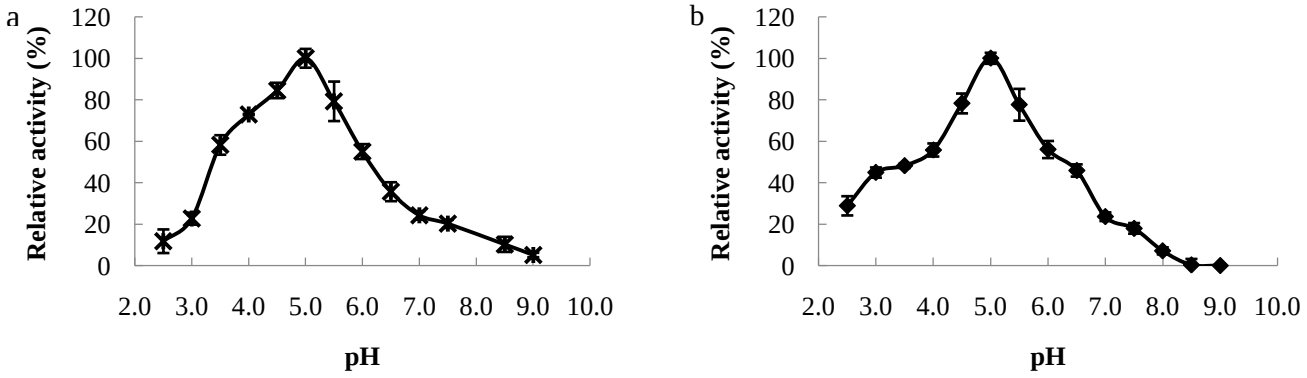

FIG. 4: EFFECT OF PH ON THE ACTIVITY AND STABILITY OF PARTIALLY PURIFIED PG (A) PH OPTIMA (B) PH STABILITY. VALUES ARE MEANS OF 3 REPLICATES WITH ± SD.

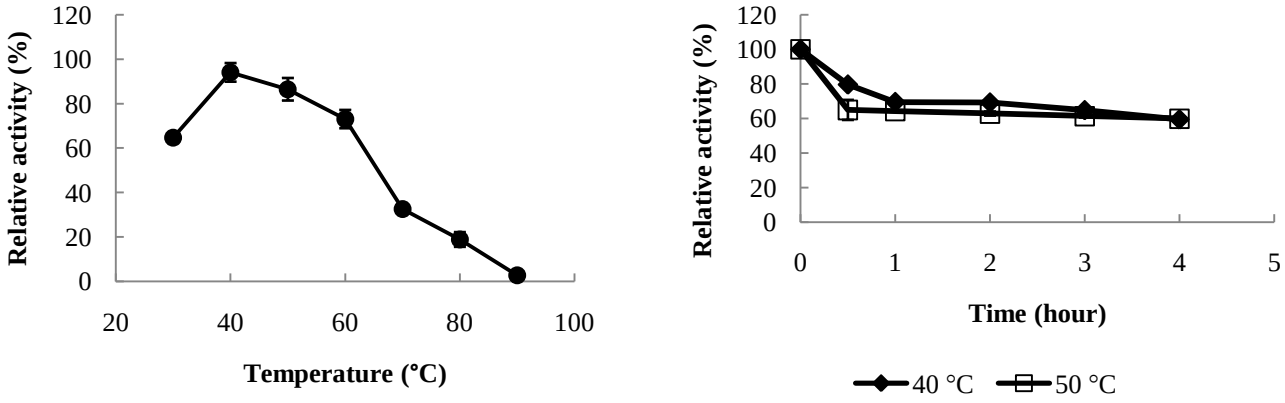

FIG. 5 EFFECT OF TEMPERATURE ON THE ACTIVITY AND STABILITY OF PARTIALLY PURIFIED PG (A) TEMPERATURE OPTIMA (B) TEMPERATURE STABILITY. VALUES ARE MEANS OF 3 REPLICATES WITH ± SD.

IV. CONCLUSION

In brief, ATPS comprising PEG and sodium citrate could potentially be used as an alternative method for purification of PG from the fermentation broth of *C. truncatum* CP2. The optimum condition for purification of PG was achieved using PEG/sodium citrate comprising crude load of 16% (w/w) at pH 7.0 with addition of 1.0% (w/w) sodium chloride. Partial purification of PG with ATPS led to 57.4% recovery of enzyme with 5.1-fold purification.

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Study of Macroinvertebrates Assemblage as an Indication of a Tropical Freshwater Lagoon Water Quality: Ono Lagoon (Côte d'Ivoire, West Africa)

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***Abstract*—** *This study aimed to assess the water quality of Ono lagoon using macroinvertebrates as bioindicators. The Biological Monitoring Working Party (BMWP) scoring system and the Pollution Tolerance Index (PTI) were used to assess the ecosystem health of water. Samples were monthly collected from September 2015 to August 2016. A total of 12145 macroinvertebrates belonging to 47 families, 17 orders, 5 classes and 3 phyla were identified. Macroinvertebrates were mainly composed of Arthropoda, Mollusca and Annelida. The most abundant taxa was Insecta (83.14%) followed by Gastropoda (6.65%) and whereas the least abundant taxa were Achaeta (6.19%), Crustacea (2.39%) and Arachnida (1.62%). The BMWP score was 140, indicating that water was neither very clean nor significantly altered aquatic habitat. According to the PTI, the water was moderately polluted based on the number of moderately pollution-sensitive organisms (52.96%) and the number of aquatic organisms which are fairly and very sensitive to pollution (34.6%). These results showed that biological quality of Ono lagoon can be considered as acceptable.*

***Keywords*— *BMWP scoring system, Macroinvertebrates, Ono Lagoon, Pollution Tolerance Index, Water quality*.**

I. INTRODUCTION

Macroinvertebrates are diverse array of animals without backbones operationally defined as those that are retained by a sieve or mesh with pore size of 0.2 to 0.5 mm, as used most frequently in stream sampling devices. Lagoon macroinvertebrates include various groups of worms, molluscs, crustaceans, mites, and above all insects (Winterbourn, 2008).

Most invertebrates are important components of lagoon ecosystems. They graze periphyton, assist in the breakdown of organic matter and cycling of nutrients and, in turn, may become food for predators (Hynes and Naba, 2012). Macroinvertebrates are the organisms most commonly used for biological monitoring of freshwater ecosystems worldwide. This is because they are found in most habitats, have generally limited mobility, are quite easy to collect by way of well established sampling techniques, and there is a diversity of forms that ensures a wide range of sensitivities to changes in both water quality (of virtually any nature) and habitats (Hellawell, 1986; Abel, 1999). Studies of aquatic ecosystems macroinvertebrates as biological monitoring techniques have been widely reported and described in the literature (Hart *et al*.,1999; Touzin, 2008; Odountan, and Abou, 2015). Macroinvertebrates assemblages have been widely used as biondicators of the overall health of different aquatic ecosystems within several biotic indices (Raburu *et al*., 2009a, 2009b). Freshwater macroinvertebrates species vary in sensitivity to organic pollution and, thus, their relative abundances have been used to make inferences about pollution loads. In Ono lagoon, increased deforestation and unsustainable agriculture coupled with agro-industrial activities pose threats to the wellbeing of aquatic ecosystems. Major industrial activities include agricultural practices of the Alsacienne Society of Côte d'Ivoire (SALCI) and the Study of Banana Crop (SCB). The proximity of leaching waters of neighbouring agricultural lands is a permanent source of pollution. Given that community livelihoods in Ono lagoon basin revolve around agricultural crop production and fisheries, it is imperative that the wetland ecosystem is closely monitored and conserved to ensure sustainability. Numerous studies have highlighted the importance of using benthic macroinvertebrates for monitoring purposes to support the results obtained regarding physical and chemical variables (Masese *et al*. 2009; Raburu *et al*. 2009a, 2009b; Minaya *et al*. 2013). These organisms were good biological indicators of water quality, due to the fact that they are both abundant and ubiquitous in nature, thereby offering a wide spectrum of observable responses to environmental changes (Turkmen and Kazanci, 2010). Till to date, no study has been undertaken to document the occurrence and distribution of macroinvertebrates assemblage in small lagoon and their response to varying levels of disturbance. The objective of this study is to present the status of water quality and ecosystem health of Ono lagoon

using the most community structure indices such as Biological Monitoring Working Party (BMWP) and Pollution Tolerance Index (PTI).

II. MATERIAL AND METHODS

2.1 Study Site

Ono lagoon (5°22'22"N and 3°33'53"W) is a small freshwater lagoon of 481 ha located in the Southeast of Côte d'Ivoire "Fig. 1". Its surface is invaded by a wide variety of habitat types such as emerged plants, free-floating macrophytes, floating leaf plants, submerged plants and white habitats, which considerably reduce the exploitable surface to 162 ha. The main macrophytes are *Echinochloa pyramidalis*, *Eichhornia crassipes*, *Salvinia molesta*, *Pistia stratiotes* and *Hydrilla verticillata*. This lagoon is irrigated by a small river (Wamon River) and connected in downstream to Comoé River. This lagoon, permanently connected to these rivers has an equatorial climate, including two rainy seasons (April-July and October-November) and two dry seasons (December-March and August-September). The permanent linkage with the Comoé River produces typical freshwater characteristics of this lagoon. Agriculture, trade, fishing and domestic wastes are the main anthropogenic activities affecting the functioning of this lagoon.

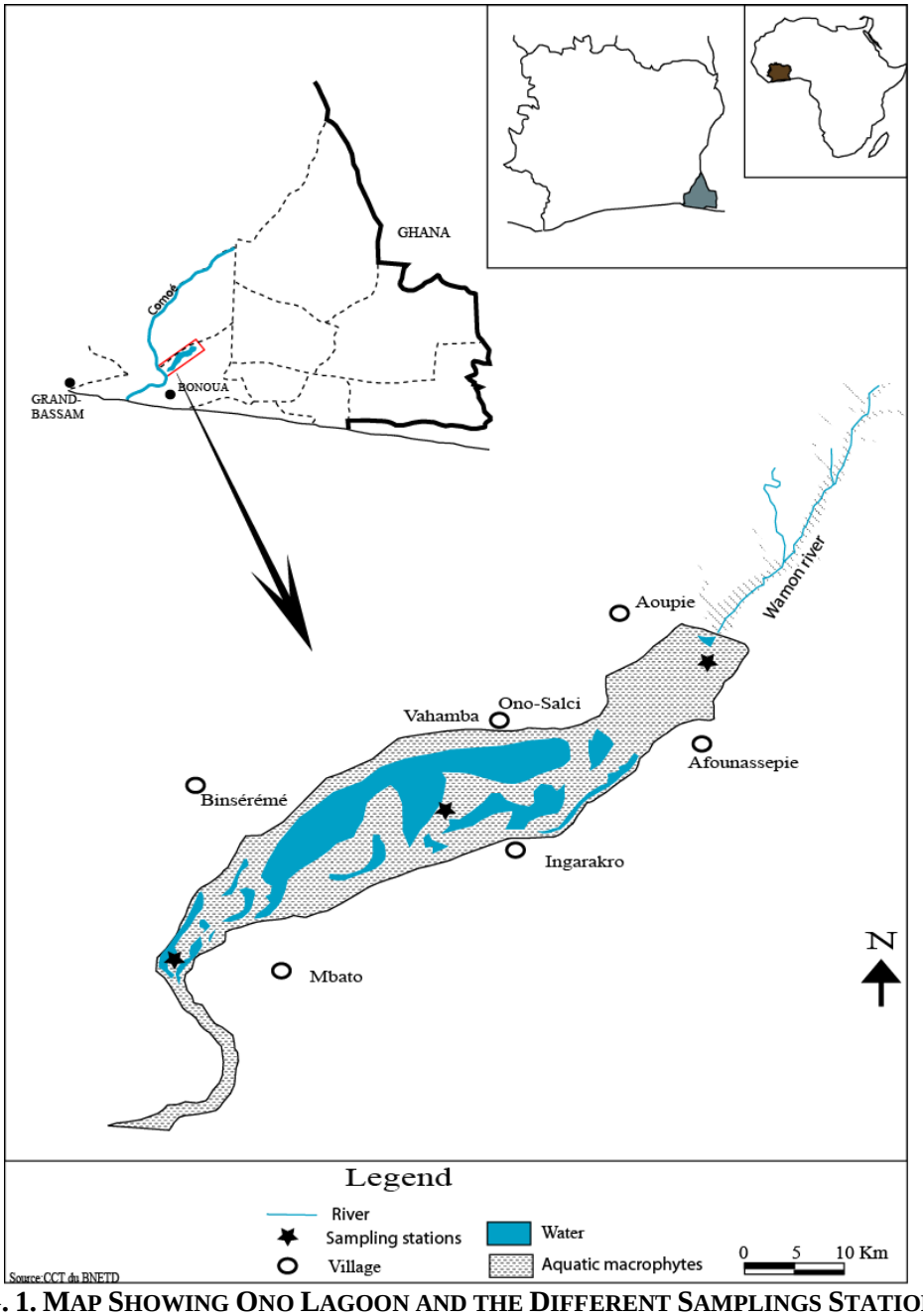

FIG. 1. MAP SHOWING ONO LAGOON AND THE DIFFERENT SAMPLINGS STATIONS

2.2 Measurement of Environmental Variables

The parameters such as transparency, water depth, pH, Total Dissolved Solids (TDS), conductivity and dissolved oxygen were recorded in *situ* between 08.00 am and 10.00 am. Temperature, pH, Total Dissolved Solid (TDS) and electric conductivity were measured using a multi parameter digital meter HANNA 9828 *vs* 2.1. Water transparency was determinate using a 20-cm-diameter Secchi disk. Water samples were taken, stored in polyethylene bottles (500 mL) and kept at a temperature below 4°C for further determination of ammonium-nitrogen (NH_4^+; mg/L), nitrate (NO_3^-; mg/L), nitrite (NO_2^-; mg/L) and phosphate (PO_4^{3-}; mg/L). The samples were filtered through Whatman GF/C fibreglass filters and concentrations were determined using a spectrophotometer Model HACH DR 6000.

2.3 Macroinvertebrates Samples

The macroinvertebrates were monthly sampled from September 2015 to August 2016 in the upstream, the middle and the downstream of the lagoon. The macroinvertebrates were collected using a van Veen grab of 0.10 m^2 internal area, a triangular hand net (10 x 10 x 10 cm, 250 µm mesh, 50 cm length) and an artificial trap (basket). Samples obtained were carefully washed through a set of sieves of mesh size 0.2 mm in the water of lagoon and the retained materiel was bottled and preserved in a 10% formaldehyde solution in a plastic container for further analysis. At laboratory, preserved samples were washed to remove formaldehyde solution and then screened through a 500 µm mesh size to collect all macroinvertebrates on white plates. They were then fixed in a 70% alcohol solution for identification. Large macroinvertebrates were sorted by the naked eye while smaller fauna was sorted under a binocular loupe. All animals were then sorted out into different taxonomic groups, counted and identified up to lowest possible taxon under binocular loupe according to the keys of (Dejoux *et al*., 1981; De Moor *et al*., 2003; Tachet *et al*., 2003; Moisan et Pelletier, 2008; Moisan, 2010).

2.4 Data analyses

The BMWP was used to determine water quality. This index requires taxonomic identification of the invertebratesto family level and order or class for some groups (Uherek and Gouveia, 2014). The analytical procedures were identification of macroinvertebrates to family level and assign them with the scores following BMWP scoring system. The BMWP score equals the sum of the tolerance scores of macroinvertebrates families in the sample (Mandaville, 2002). A higher BMWP score is considered to reflect a better water quality (Aquilina, 2013). Alba-Tercedor (1996) and Armitage *et al*. (1983) claimed that the total score for a site indicates water quality categories ranging from good to very critical (as cited in Uherek and Gouveia, 2014). Each taxon receives a score that reflect site exposure to pollution; that is, pollution-sensitive taxa receive high scores, while pollution-tolerant taxa are given low scores (Suleiman and Abdullahi, 2011). Table 1 presents BMWP taxa scores (class, order, or family) where each family is given a score between 1 and 10 according to the presence or absence of indicator groups and indicator species in the sample (Uherek and Gouveia, 2014). Table 2 presents class, scores, categories and interpretation of the result that used to classify the water quality of Ono lagoon based on BMWP score system (Uherek and Gouveia, 2014; Junqueira and Campos, 1998). The Pollution Tolerance Index (PTI) is currently used by all Pennsylvania volunteer citizen monitoring groups and the Department of Environmental Protection for their stream organism sampling. It is based on the concept of indicator organisms and tolerance levels. The procedures are designed to be done quickly and easily; they provide a rapid means of sampling riffle and other shallow areas to detect moderate to severe stream quality degradation.

TABLE 1
THE BIOLOGICAL MONITORING WORKING PARTY SCORE (BMWP) TAXA SCORES: CLASS, ORDER, OR FAMILY

Taxa	Score
Ephemeroptera: Leptoplebiidae, Leptohyphidae **Plecoptera:** Perlidae **Trichoptera:** Brachycentridae, Leptoceridae, Odontoceridae and Secicostomatidae	10
Odonata: Coenagrionidae, Calopterygidae, Cordulegasteridae, Gomphidae and Libellulidae **Trichoptera:** Calamoceratidae, Glossosomatidae, Philopotamidae and Psychomyiidae	8
Plecoptera: Nemouridae **Trichoptera:** Polycentropodidae	7
Crustacea **Trichoptera:** Hydrobiosidae, Hydroptilidae	6
Coleoptera: Elmidae, Dryopidae **Diptera:** Simuliidae, Tipulidae **Ephemeroptera:** Euthyplociidae, Polymitarcidae **Platyhelminthes** **Trichoptera:** helicopsychidae, Hydropsychidae	5
Arachnida: Hydracarina **Coleoptera:** Chrysomelidae, Curculionidae and Haliplidae **Diptera:** Anthomyiidae, Ceratopogonidae, Chaoboridae, Dixidae, Dolichopodidae, Empididae, Limoniidae, Psychodidae, Stratiomyidae and Tabanidae **Ephemeroptera:** Baetidae, Caenidae **Megaloptera:** Corydalidae, Sialidae	4
Annelida: Hirudinea **Coleoptera:** Dytiscidae, Gyrinidae, Helodidae, Hydrophilidae and Noteridae **Hemiptera:** Belostomatidae, Corixidae, Gerridae, Hydrometridae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae, Pleidae and Veliidae **Mollusca**	3
Diptera: Chirominidae, Culicidae, Ephydridae, Muscidae and Thaumaleidae	2
Annelida: Oligochaeta **Blattaria:** Blattidae **Diptera:** Sciomyzidae, Syrphidae and Rhagionidae **Lepidoptera**	1

Source: Uherek and Gouveia (2014).

TABLE 2
VALUES FOR INTERPRETING THE RESULTS OF BMWP INDEX.

CLASS	BMWP score	CATEGORY	INTERPRETATION	COLOR
I	> 150 101 – 150	Good	Very clean (pristine waters) Clean or not significantly altered	Blue
II	61 – 100	Acceptable	Clean but slightly impacted	Green
III	36 – 60	Questionable	Moderately impacted	Yellow
IV	15 – 35	Critical	Polluted or impacted	Orange
V	< 15	Very critical	Heavily polluted water (seriously affects system)	Red

Source: Junqueira and Campos (1998) and Uherek and Gouveia(2014).

III. RESULTS

3.1 Physical and chemical variables

The variations of environmental parameters are given in Table 3. Water temperature ranged between 25.86±0.71°C (low dry season) and 27.82±1.49°C (high dry season). The lowest dissolved oxygen values were recorded in high dry season (1.56±1.15 mg/L) and the highest values were observed in low rainy season (3.25±3.22 mg/L). The electric conductivity varied from 13.77±9.09 µS/cm (low dry season) to 21.60±2.25 µS/cm (low rainy season). High dry season presented low values of pH (5.60±0.16) while high values (7.01±0.46) were recorded in high rainy season. Water transparency fluctuated between 0.89±0.05 cm (low rainy season) and 2.03±0.12 cm (low dry season). Total Dissolved Solid value was higher in low rainy season (10.83±1.07 mg/L) and lower in low dry season (6.88±4.67 mg/L). Water deep was more important in low rainy season (2.75±0.21 m). Nitrite values varied between 0.01±0.00 mg/L (low dry season) and 0.45±0.64 mg/L (high dry season). Phosphate oscillated between 0.39±0.32 mg/L (low dry season) and 0.53±0.83 mg/L (low rainy season). Ammonium value varied from 0.05±0.05 mg/L (high dry season) to 0.12±0.13 mg/L (low dry season). Nitrate value was higher in low dry season (3.76±1.50 mg/L). The variables such as pH, electric conductivity, Total Dissolved Solid, water transparency and water deep showed significative difference between the different seasons.

TABLE 3
PHYSICOCHEMICAL CHARACTERISTICS (MEAN ± (SD)) OF ONO LAGOON AT VARIOUS SEASONS ; HDS : HIGH DRY SEASON ; HRS : HIGH RAINY SEASON ; LDS : LOW DRY SEASON ; LRS : LOW RAINY SEASON.

Parameters	Seasons			
	HDS	HRS	LDS	LRS
Temperature (°C)	27.82±1.49[a]	26.98±1.65[a]	25.86±0.71[a]	27.60±1.49[a]
Dissolved Oxygen (mg/L)	1.56 ±1.15[a]	2.44±1.40[a]	2.47±1.72[a]	3.25±3.22[a]
pH	5.60±0.16[a]	7.01±0.46[b]	6.58±1.06[b]	6.11±0.44[a]
Conductivity (µS/cm)	21.37±3.62[b]	15.19±4.58[a]	13.77±9.09[a]	21.60±2.25[b]
TDS (mg/L)	10.76±2.25[b]	7.49±2.34[a]	6.88±4.67[a]	10.83±1.07[b]
Transparency (m)	1.91±0.49[b]	1.33±0.41[a]	2.03±0.12[b]	0.89±0.05[a]
Water Deep (m)	2.36±0.10[a]	2.53±0.08[a]	2.19±0.25[a]	2.75±0.21[b]
Nitrite (NO2) (mg/L)	0.45±0.64[a]	0.17±0.29[a]	0.01±0.00[a]	0.02±0.03[a]
Nitrate (NO3) (mg/L)	2.53±0.52[a]	3.71±1.44[a]	3.76±1.50[a]	2.29±0.89[a]
Ammonium (mg/L)	0.05±0.05[a]	0.07±0.04[a]	0.12±0.13[a]	0.10±0.06[a]
Phosphate (mg/L)	0.46±0.33[a]	0.49±0.20[a]	0.39±0.32[a]	0.53±0.83[a]

[a,b]: letters showed the difference between the seasons as regards the parameter indicated.

3.2 Macroinvertebrates composition

A total of 12145 macroinvertebrates belonging to 47 families, 17 orders, 5 classes and 3 phyla were collected and identified in Ono lagoon. The phyla were Arthropoda, Mollusca and Annelida while the classes were Insecta, Crustacea, Arachnida, Gastropoda and Achaeta. The most diverse class was Insecta with 6 orders and 29 families. The most abundant taxa was Insecta (83.14%) followed by Gastropoda (6.65%) and Achaeta (6.19%) whereas the least abundant taxa were Crustacea (2.39%) and Arachnida (1.62%). The group of Insecta was mainly composed of Hemiptera (36.28%), Coleoptera (21.52%), Diptera (21.11%) and Odonata (18.27%) representing about 97.19% of the total abundance. Ephemeroptera and Megaloptera are presented about 2.81% of total Insecta.

3.3 Ono lagoon water quality

The aquatic macroinvertebrates collected from Ono lagoon and the biological scores allocated to each family of aquatic macroinvertebrates are presented in Tables 4 and 5. These scores present the presence of indicator groups and indicator species in the sample. The total BMWP score of Ono lagoon is 140 (Table 5), indicating that this lagoon is within class I (101-150) represented the category of good with the interpretation of clean or not significantly altered aquatic environment (Table 2 and Table 6). The analysis of PTI breaks **macroinvertebrates** into 4 groups (Table 7): **Group I was composed by** Intolerant to pollution **(**Riffles beetles, Mayfly larva, Caddisflies, Dobsonflies, Gilled Snails **), Group II was** Moderately intolerant to pollution (Dragonfly, Damselfly, Craneflies, Water scavenger beetles, Water scavenger beetles, Predaceous diving beetles, Leaf Beetles, Shrimps), Group III was Fairly tolerant to pollution (Midges) and Group IV was Very tolerant to pollution **(**Syrphid Flies, Worm**).** Moderately intolerant to pollution group was more abundant (52.96%) and Very tolerant to pollution was less abundant (8.46%). Fairly tolerant to pollution and Very tolerant to pollution represented 34.6% of total abundance.

TABLE4

TAXA OF AQUATIC MACROINVERTEBRATES COLLECTED FROM ONO LAGOON AND THEIR ABUNDANCE.

Phyla	Classes	Orders	Families	Abundance
Arthropoda	Insecta	Heteroptera	Belostomatidae	1326
			Naucoridae	580
			Hydrometridae	89
			Notonectidae	556
			Gerridae	333
			Corixidae	512
			Veliidae	31
			Mesovellidae	70
			Nepidae	167
		Diptera	Chironomidae	1760
			Tabanidae	23
			Ceratopogonidae	13
			Culicidae	311
			Syrphidae	15
			Tipulidae	10
		Coleoptera	Hydrophilidae	974
			Dytiscidae	841
			Helodidae	45
			Chysomelidae	37
			Curculionidae	150
			Elmidae	126
		Odonata	Libellulidae	935
			Corduliidae	362
			Aeshnidae	42
			Cordulegasteridae	6
			Coenagrionidae	500
		Ephemeroptera	Baetidae	247
		Trichoptera	Hydroptilidae	31
		Megaloptera	Corydalidae	6
	Crustacea	Amphipoda	Gammaridae	7
		Decapoda	Atyidae	38
			Crangonidae	78
			Penaeidae	167
	Arachnida	Aranae	Tetragnatidae	15
			Pissauridae	47
Mollusca	Gastropoda	Mesogastropoda	Ampullariidae	125
			Physcidae	281
			Hydrobiidae	28
		Basommatophore	Planorbidae	354
			Lymnaeidae	20
Annelida	Achaeta	Oligochaeta	Oligochaeta	234
			Tubificidae	325
		Clitellata	Haplotaxidae	102
			Lumbricidae	51
		Pharyngobdelliforme	Herpodelidae	31
		Rhynchobdellida	Glossiphoniidae	9
Total=3	**5**	**17**	**47**	**12145**

TABLE 5
BIOLOGICAL SCORES ALLOCATED TO GROUPS OF AQUATIC MACROINVERTEBRATES COLLECTED FROM ONO LAGOON.

Phyla	Classes	Orders	Families	Scores
Arthropoda	Insecta	Heteroptera	Belostomatidae	3
			Naucoridae	3
			Hydrometridae	3
			Notonectidae	3
			Gerridae	3
			Corixidae	3
			Veliidae	3
			Mesovellidae	3
		Diptera	Chironomidae	2
			Tabanidae	4
			Ceratopogonidae	4
			Culicidae	2
			Syrphidae	1
			Tipulidae	5
		Coleoptera	Hydrophilidae	3
			Dytiscidae	3
			Chrysomelidae	4
			Curculionidae	4
			Elmidae	5
		Odonata	Libellulidae	8
			Cordulegastridae	8
			Coenagrionidae	8
		Ephemeroptera	Baetidae	4
		Trichoptera	Hydroptilidae	6
		Megaloptera	Corydalidae	4
	Crustacea	Amphipoda	Gammaridae	6
		Decapoda	Atyidae	6
			Crangonidae	6
			Penaeidae	6
Mollusca	Gastropoda	Mesogastropoda	Ampullariidae	3
			Physidae	3
			Hydrobiidae	3
		Basommatophore	Planorbidae	3
			Lymnaeidae	3
Annelida	Achaeta	Oligochaeta	Oligochaeta	1
			Tubificidae	1
Total				**140**

TABLE6
VALUE OF BMWP SCORE OBTAINED WITH MACROINVERTEBRATE COLLECTED IN ONO LAGOON AND IT INTERPRETATION.

CLASS	CATEGORY	BMWP score	INTERPRETATION	COLOR
I	GOOD	140	Clean or not significantly altered	

TABLE 7
FOUR GROUPS OF MACROINVERTEBRATES BASED ON WATER POLLUTION TOLERANCE: INTOLERANT TO POLLUTION, MODERATELY INTOLERANT TO POLLUTION, FAIRLY TOLERANT TO POLLUTION AND VERY TOLERANT TO POLLUTION ACCORDING TO LEWIS (2014).

Group	Collected macroinvertebrates	Total number of taxa	Abundance in %
Intolerant to pollution	Riffles beetles	126	1.86
	Mayfly larva	247	3.64
	Caddisflies	31	0.46
	Dobsonflies	6	0.09
	Gilled Snails	434	6.40
Total		**844**	**12.44**
Moderately intolerant to pollution	Dragonfly	941	13.87
	Damselfly	500	7.37
	Craneflies	10	0.15
	Water scavenger beetles	974	14.36
	Predaceous diving beetles	841	12.40
	Leaf Beetles	37	0.55
	Shrimps	290	4.27
Total		**3593**	**52.96**
Fairly tolerant to pollution	Midges	1773	26.14
Total		**1773**	**26.14**
Very tolerant to pollution	Syrphid Flies	15	0.22
	Worm	559	8.24
Total		**574**	**8.46**
Total number of all macroinvertebrates		**6784**	

IV. DISCUSSION

This study showed that several species of aquatic macroinvertebrates belonging to 47 families, 17 orders, 5 classes and 3 phyla (Arthropoda, Mollusca and Annelida) colonize Ono lagoon. Arthropoda, Mollusca and Annelida were generally collected in lagoon (Berame, 2017). Insecta was qualitatively and numerically the most dominant group among macroinvertebrates. This finding is similar to previous studies in which Insecta was found to be the most dominant group in some streams (Türkmen and Kazanci, 2010; Kalyoncu and Zeybek, 2011; Zeybek *et al*., 2014). Based on the BMWP score, the ecosystem health of Ono lagoon can be placed into category ”Good”, indicating clean or not significantly altered aquatic environment. Since the BMWP score lay between 101 and 150, it can be interpreted as "Clean or not significantly altered" aquatic ecosystem. This means that water of Ono lagoon is not very clean (>150). This interpretation is mirrored by large number of moderately intolerant to pollution organisms (52.96%) as well as fairly tolerant to pollution and Very tolerant to pollution organisms which represented 34.6% of macroinvertebrates abundance. Similarly, the stream is not significantly altered because it supports some macroinvertebrates individuals which are sensitive to pollution (Czerniawska-Kusza, 2005; Bonada *et al*., 2006). Therefore, water of Ono lagoon can be interpreted as being moderately polluted based on the number of moderately pollution-sensitive organisms and BMWP score (Sandin and Hering, 2004). This pollution can be due to agricultural activities developed nearly the stream. Agricultural activities alter physico-chemical parameters of the stream

and hence changing the abundance of macroinvertebrates as well as the quality of water (Dahl *et al*., 2004; Ojija, 2015). Farming activities nearly the stream causes soil erosion and consequently increasing suspended particles into the stream. Farming that employs the use of synthetic fertilizers, pesticides, and weedicides, and settlements that demand space are another factors contributing to pollution of the aquatic environment of Ono lagoon (Ojija and Laizer, 2016). Kripa *et al*. (2013) argued that human intervention in the name of development has largely affected many natural aquatic ecosystems over the world.

V. CONCLUSION

In conclusion, the present study reports 47 macroinvertebrates famillies belonging to 3 phyla, 5 classes and 17 orders in the different inventoried stations. Insecta was the most diversified group. This group was numerically the most abundant in Ono lagoon. The calculated total BMWP score of Ono lagoon is 140. Ono lagoon is in class I, category of good with the interpretation of clean or not significantly altered aquatic environment. According to the Pollution Tolerance Index, the water of Ono lagoon can be interpreted as being moderately polluted. This water is not very clean, but it biological quality can be considered as acceptable. However, the anthropogenic activities should be controlled and the Ono lagoon regularly monitored by the relevant authorities.

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"Hydroponic hop crop (*Humulus lupulus* L.) under greenhouse conditions in Mexico City".

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***Abstract*—** *H. lupulus is a dioecious plant member of the Cannabaceae family whose female flowers are used in the brewing industry. The value of female plants lies in the lupulin glands that contain resins and essential oils required for the flavor and aroma of beer. Hop crop growths between 35 ° and 55 ° latitude north and south, however, it has been possible to cultivate where conditions does not resemble those observed in the mentioned latitudes. Even more, hop has been hydroponic cultivated in traditional crop areas. Hydroponics provides controlled conditions, isolation and all needed nutrients in an aqueous solution. The aim of this work was to establish a hydroponic hop culture in greenhouse conditions, using a modified Hoagland nutrient solution. An average daily growth rate of 1.17 cm was obtained by rhizome plants and of 1.38 cm for those from freshly germinated seedlings, and an average overall height of 218 cm, an adequate growth when compared to the background of this crop. The data obtained show that hydroponics hop crops can be settle in areas with different conditions from those of the traditional cultivation zone.*

***Keywords*— Brewing industry, hop, hydroponics, rhizome, seedling.**

I. INTRODUCTION

H. lupulus is a dioecious plant member of the Cannabaceae family whose female flowers are used in the brewing industry. The cultivated hop, a short day, climbing, herbaceous plant produces new shoots in early spring and senesces to the perennial rootstock in autumn [1]. The value of female plants lies in the lupulin glands that contain resins and essential oils required for the flavor and aroma of beer [2, 3].

Hops are native to the regions of North America, Europe and Asia. It has been used in brewing for hundreds of years, the oldest crop known to date 500 years ago in central Europe [4]. Currently, the main producers are: Germany, the United States and China [5]. One of the most important places for hops cultivation is Hallertau, Germany, which has a Dfb climate (hemiboreal without dry season, mild summer and cold winter), according to the Köppen classification [6], these climatic conditions favor the growth of hops and for that reason, the main producers are areas with similar conditions. Due to the high demand of these flowers, it is now possible to find traditional hop crops in places with different conditions in countries such as Argentina, Chile and Mexico; while hydroponic crops, in addition to the first ones, that were carried out in Armenia, are also commercially developed in Colorado, United States [7, 8, 9, 10, 11, 12, 13].

Like any plant, hops need nutrients from the soil to develop, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur that requires in large quantities (more than 1000 mg/kg of dry matter); and iron, manganese, zinc, boron, molybdenum, nickel, copper and chlorine, which the plant needs in small quantities (less than 100 mg/kg of dry matter) [14, 15, 16]. For the hops plant, the most critical macronutrients are potassium and nitrogen. Regarding micronutrients, hops are negatively impacted considerably by deficiencies of boron and zinc [17]. In the hydroponic culture established in Armenia, the nutrient solution of Davtyan was used: 311 ppm of nitrogen, 65 ppm of phosphorus, 350 ppm of potassium, 150 ppm of calcium and 30 ppm of magnesium as main nutrients [12, 18]. This technique could allow to establish crops in other places where there is a growing demand of high quality and inexpensive hops but the conditions are not favorable, as it is Mexico City [8, 19, 20, 21, 22, 23]. So, the aim of this work was to establish hydroponic hop culture in greenhouse conditions, using a modified Hoagland nutrient solution.

II. MATERIAL AND METHOD

Hop seedlings of the Cascade variety were kept in soil for three months and then each placed in a 1 liter glass with substrate (50% vermiculite and 50% agrolite) [24] and modified Hoagland nutrient solution "A" (Table 1). After 27 days, the plants were moved to larger containers (8L). Hop rhizomes of the same variety were also placed with the same treatment. After 107 days in "A" solution, both the seedlings and the rhizome plants were watered with the modified Hoagland nutrient solution "B" (Table 1). During the entire growth time (plant from seedlings 207 days and plant from rhizome 183 days), 2

measurements were taken a week from the length of the stem. A Student's t test was applied comparing the final size and the daily growth rate between rhizome plants and seedlings plants [25].

TABLE 1
NUTRIENT SOLUTION USED IN THIS WORK [28, 29, 30]

Element	Concentration		
	Hoagland	Hoagland modified "A"	Hoagland modified "B"
Nitrogen (NO_3 -N)	210 ppm	182.45 ppm	148.44
Phosphorus (P)	31 ppm	31 ppm	66.36
Potassium (K)	235 ppm	235 ppm	173.14
Sulfur (SO_4 - S)	64 ppm	71 ppm	71 ppm
Calcium (Ca)	200 ppm	283 ppm	283 ppm
Magnesium (Mg)	48 ppm	125 ppm	125 ppm
Iron (Fe)	1-5 ppm	12 ppm	12 ppm
Chlorine (Cl)	95 ppm	176 ppm	176 ppm
Manganese (Mn)	0.5 ppm	7 ppm	7 ppm
Boron (B)	0.5 ppm	2 ppm	2 ppm
Zinc (Zn)	0.05 ppm	7 ppm	7 ppm
Copper (Cu)	0.02 ppm	2 ppm	2 ppm
Molybdenum (Mo)	0.01 ppm	2 ppm	2 ppm

The last 90 days the plants were subjected to the photoperiod of Hallertau, Germany, beginning with 16 light hours and decreasing weekly 15 minutes the first four weeks, 20 the second four and 30 the last four weeks [26].

III. RESULTS AND DISCUSSION

The daily growth rate of the plants from the rhizome was 1.17 cm; In the case of plants from seed, the daily growth rate was 1.38 cm (figures 1, 2, 3). The Student t test with a confidence interval of 95% showed that there is no statistically significant difference between the two samples, in the same way the final average height reached by each group was 2.18 m and 3.048 m respectively for which there was no statistically significant difference.

FIG. 1. PLANTS FROM RHIZOME AND SEEDLINGS AT THE END OF THE EXPERIMENT.

Hops plants grew considerably under hydroponic conditions, reaching an overall average height of 2.54 m, but taller than two of varieties used by Pearson and collaborators [27], who reported average heights of 6.09 m, 4.89 m, 2.29 m and 2.21 m for the Chinook, Columbus, Amalia and Neo1 varieties respectively. There are commercially productive hop crops of Amalia and Neo1 varieties in the southeastern part of USA, even with these sizes.

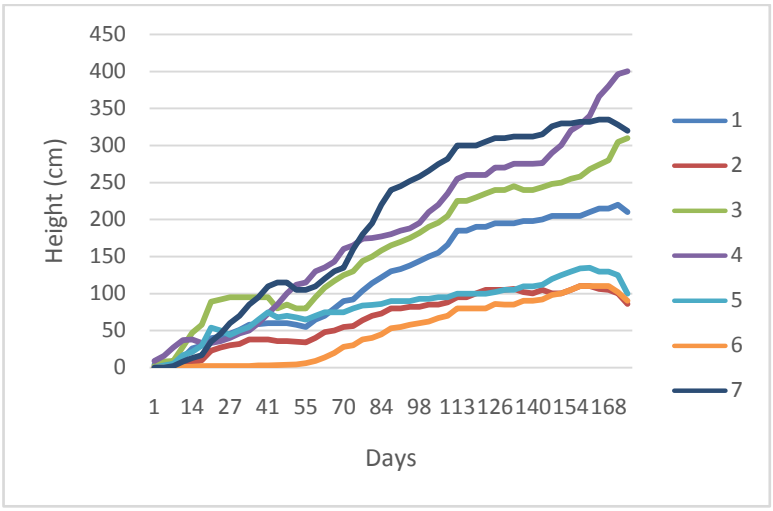

FIG. 2. GROWTH OF *H. LUPULUS* PLANTS FROM RHIZOMES.

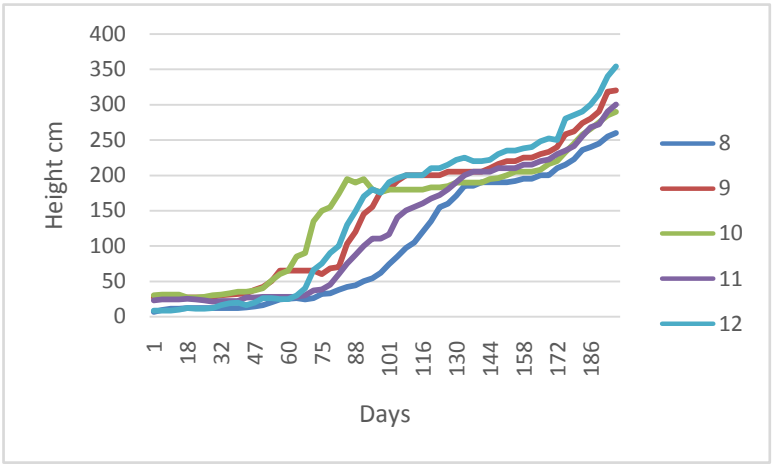

FIG. 3. GROWTH OF *H. LUPULUS* PLANTS FROM SEEDLINGS.

In contrast, hop plants grown in hydroponic culture [12], reached an average height of 4.3 m, however it is important to consider that Armenia is located in the latitudes corresponding to the traditional growing area.

IV. CONCLUSION

The results of this work support the establishment of hydroponic hop crops in places where conditions are not favorable, such as Mexico City, where there is a growing demand for high quality hops at a reasonable price.

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Determining the best Lentil (*Lens culinaris*) and Mustard (*Brassica campestris*) Intercrop Combination to Improve Biomass Yield and Economic Returns on the Yield in Southern Region of Bangladesh

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Abstract— *An intercropping experiment on mustard with lentil was conducted during rabi season of 2013-14 and 2014-15 at the Regional Agricultural Research Station, Rahmatpur, Barisal to find out suitable intercrop combination for higher profitability and economic return. Sole lentil (100%), sole mustard (100%) and five intercrop combinations {one row of lentil and one row of mustard (1:1), one row of mustard in between two rows of lentil (2:1), one row of mustard in between three rows of lentil (3:1), one row of mustard in between four rows of lentil (4:1) and two rows of mustard in between four rows of lentil (4:2)} were evaluated in the present study. Significantly the highest lentil (1.91 t/ha) and mustard yield (1.48 t/ha) were obtained from their respective sole crops. Lentil yield was reduced (17-34%) due to intercropping, but it was compensated by the mustard yield. Moreover, land equivalent ratios (LER) of the intercrop treatments were higher than those of sole crops. The highest (2.29t/ha) lentil equivalent yield (LEY) was obtained from T_5 treatment (One row of lentil + Three rows of mustard). This treatment also gave the highest LER (1.65), gross return (Tk 182400/ha), net return (Tk. 103200/ha) and benefit cost ratio (2.29).*

Keywords— Corroboration, Lentil (*Lens culinaris*), Mustard (Brassica campestris), Intercropping and Equivalent yield.

I. INTRODUCTION

Intercropping is a traditional practice in Bangladesh. It increases total productivity per unit area through maximum utilization of land, labor and growth resources. Growing two or more crops in combination is called intercropping where intra and inter specific competition occurs (Spilter, 1983). The success of intercropping thus depends mostly on the complementary or competitive behaviors (Nazir *et al*., 1988, Chandrasekhar *et al.*,1988) of the component crops. Competitiveness of component crops depends to a large degree on each crops response to the limiting factors (Fukai and Trenbath, 1993). Therefore, intercropping is considered to be very efficient technique in maximizing the production per unit area and it gives higher resource use efficiency (Hashem and Moniruzzaman, 1986; Quayyum *et al.*,1999). It also increases land equivalent ratio (LER) to varying degrees (Mehta and De, 1980; Hashem *et al.*,1990). Lentil and mustard two most important crops in Bangladesh are usually grown as sole and intercrop. Lentil is one of most valuable cash crop grown for domestic as well as export purpose. By adopting appropriate planting geometry in the intercropping system,the total productivity can be increased (Umrani *et al*., 1984). Successful intercropping system gives higher cash return total production per unit area and diversifies production system than growing sole crop (Kurata 1966; Khaliq *et al.*,1997) and provides greater resource use efficiency (Pathic&Mulla, 1979). Several workers also reported higher economic advantage in intercropping than sole cropping (Mandal*et al.*,2004; Khaliq *et al.*,1997; Hossain and Bari, 1996). Proper row arrangement of lentil and mustard under intercropping system can ensure higher productivity and economic return. The review of research work done so far indicated that growth of mustard as intercrop is more beneficial than growing lentil alone in many situations (Aravazhi *et al*., 1997; Natarajan, 1992, Sadashiv, 2004). However, literature relating optimum plant population of mustard for intercropping with lentil is meager. Hence this experiment was undertaken to find out the optimum plant population of mustard in association with lentil for achieving higher productivity.

II. MATERIALS AND METHODS

The experiment was conducted during the rabi season of 2013-14 and 2014-15 at the Regional Agricultural Research Station, Rahmatpur, Barisal. The soil was sandy loam having pH 7.5 of Agro Ecological Zone 12. The soil was low in organic matter (1.34 %) and deficient in total nitrogen (0.05%), available phosphorus (12 µg/g soil), exchangeable potassium (0.09 meq/100 g soil) and available sulphur (2.2 µg/g soil). The unit plot size was 4m x 4 m. Five treatment combinations viz., T_1= Sole lentil (30 cm x 20 cm), T_2 = Sole mustard (30 cm x 20 cm), T_3 =1 row lentil + 1 row of mustard,T_4=1 Row mustard between every two rows of lentil (2:1), T_5 =1 row of mustard between every 3 rows of lentil (3:1) ,and T_6 =1 row mustard between every four rows of lentil (4:1), T_7 =2 rows of mustard between every 4 rows of lentil (4:2). The experiment was tested in a randomized complete block design with three replications. The seeds of lentil (BARI Mosur 6) and were mustard (BARI Sarisha 15) sown on November 19, 2014. Lentil sole and intercrop plots had received a uniform application of 50-90-40-102 kg NPKS /ha and sole mustard plot had received 250-175-90-160 kg NPKS /ha through urea, triple super phosphate (TSP), muriate of potash (MoP) and gypsum. In lentil and intercrop plots, all fertilizers were applied as basal. But in case of sole mustard plot, one half of urea and full amount of TSP, MoP and gypsum were applied as basal and remaining half ureawere applied before flowering at 30 days after sowing (DAS). Cow dung (5 t/ha) were applied as basal in all the plots. Intercultural operations like weeding, irrigation and plant protection measures were provided to both the crops as and when necessary. Lentil was harvested on 11 March 2015 (120 Days after sowing). Lentil and mustard were harvested as whole plot basis. At harvest, 10 randomly selected plants of each crop from each plot were uprooted for the assessment of yield components. The collected data were analyzed statistically and the means were adjudged using LSD. Economic analysis and benefit cost ratio (BCR) were also computed.

III. RESULTS AND DISCUSSION

3.1 Yield and yield attributes of lentil

Treatments had significant effects on pods and siliqua per plant, seeds per fruit, yield per plant (Table 2 and Table 3). Plant height does not show significant variation among the treatments. Significantly the maximum number of pods per plant (117.80 and 121.63) was observed in T_1 treatment followed by T_3 and T_5 treatment. The least number of pods per plant (66.43 and 69.33) was obtained from T_7 treatment. Shading due to higher population probably resulted in less number of fruits/plant in T_4 treatment. Significantly the maximum 1000 seeds weight (23.5 and 24 g) were obtained from sole lentil which was statistically similar to T_3 treatment and lowest 1000 seeds weight (18.27 and18.15g) were found in T_7 treatment and Significantly the maximum yield (1.91 and 1.99 tha^{-1}) was obtained from sole lentil and lowest was obtained from T_7 (1.27 and 1.29tha^{-1}) treatment that might be due to competition in this treatment. The yield of lentil in intercropping situation was reduced by 17-34% at various treatments.

TABLE 1
YIELD AND YIELD ATTRIBUTES OF LENTIL AS AFFECTED BY INTERCROPPING WITH MUSTARD WITH MUSTARD DURING RABI SEASON OF 2013-14

Treatment	Plant height (cm)	No.of pod/plant	1000 seed weight (g)	Seed yield (tha^{-1})
T_1	41.93	117.80	23.50	1.91
T_3	34.27	84.07	22.67	1.46
T_4	37.80	92.67	20.00	1.52
T_5	33.67	106.70	20.33	1.61
T_6	34.33	76.53	19.33	1.40
T_7	39.13	66.43	18.17	1.27
LSD(0.05)	NS	9.82	5.262	0.56
CV%	8.91	7.10	12.68	2.87

TABLE 2

YIELD AND YIELD ATTRIBUTES OF LENTIL AS AFFECTED BY INTERCROPPING WITH MUSTARD DURING RABI SEASON OF 2014-15

Treatment	Plant height (cm)	No. of pod/plant	No. 1000 seed weight (g)	Seed Yield (tha^{-1})
T1	43.33	121.63	24.00	1.99
T3	36.07	82.83	21.00	1.43
T4	37.56	91.08	22.00	1.49
T5	40.29	108.35	20.34	1.72
T6	34.47	77.53	19.63	1.37
T7	33.45	69.33	18.15	1.29
LSD(0.05)	4.95	10.99	2.25	0.47
CV%	7.63	7.89	10.28	2.64

Where, T_1=Sole Lentil, T_3= One row of Lentil+One row of mustard, T_4=One row of mustard in between every two rows of lentil, T_5= One row of mustard in between alternate three rows of lentil, T_6=One row of mustard in between every four rows of lentil and T_7=Two rows of mustard in between every four rows of lentil

3.2 Yield and yield attributes of mustard

Plant height does not show significant variation among the treatments. But intercropping has significant effect on the yield and yield attributing characters of mustard. The highest no. of siliqua (119 and 117.33) per plant, 1000 seed weight (3.90 and 2.71g) was observed in T_2 treatment which was followed by T_3 treatment. Significantly highest yield (1.48 and 1.52 t/ha) of mustard was obtained from sole mustard and lowest from T_7 treatment (Table 3 and Table 4). The yield of mustard in intercropping situation was reduced by 13-41% at various treatments.

TABLE 3

YIELD AND YIELD ATTRIBUTES OF MUSTARD AS AFFECTED BY INTERCROPPING WITH LENTIL DURING RABI SEASON OF 2013-14

Treatment	Plant height (cm)	No. of siliqua/plant	1000 seed weight (g)	Yield (tha^{-1})
T_2	84.13	119.00	3.41	1.48
T_3	88.93	103.90	3.37	1.33
T_4	89.60	89.13	3.32	1.29
T_5	92.33	83.53	3.39	1.25
T_6	83.63	77.23	3.34	1.22
T_7	88.53	68.73	3.29	1.15
LSD (0.05)	NS	8.95	NS	0.06
CV%	10.75	5.63	1.21	2.73

TABLE 4

YIELD AND YIELD ATTRIBUTES OF MUSTARD AS AFFECTED BY INTERCROPPING WITH LENTIL DURING RABI SEASON OF 2014-15

Treatment	Plant height (cm)	No. of siliqua/plant	100 seed weight (g)	Yield (tha^{-1})
T_2	84.12	118.02	2.79	1.61
T_3	83.54	117.33	2.71	1.52
T_4	89.43	121.50	2.65	1.36
T_5	82.65	108.35	2.58	1.27
T_6	86.13	105.33	2.62	1.21
T_7	83.36	98.50	2.69	1.14
LSD(0.05)	NS	9.05	NS	0.75
CV%	9.17	6.19	1.13	2.52

Where, T_1=Sole Lentil, T_3= One row of Lentil+One row of mustard, T_4=One row of mustard in between every two rows of lentil, T_5= One row of mustard in between alternate three rows of lentil, T_6=One row of mustard in between every four rows of lentil and T_7=Two rows of mustard in between every four rows of lentil.

3.3 Economic analysis

Productivity of lentil + mustard intercropping system was evaluated on the basis of equivalent yield (Bandyopadhyay 1984). All the intercropped combinations showed the higher lentil equivalent yield over sole lentil except T_7 treatment (Table 5 and

Table 6). Among the intercrop treatments, the highest lentil equivalent yield (2.28 t/ha) was obtained from T_3 and the lowest (1.84t/ha) from T_7 treatment. Highest LER (1.65) was found in T_3 treatment followed by T_5. Intercropping showed higher gross return than sole cropping. Although higher cost of production was involved in intercropping system, highest gross return (Tk152400/ha) and net return (Tk103200/ha) was obtained from T_5 treatment. Moreover, the treatment T_3 contributed to the highest benefit cost ratio (2.29). The results revealed that mustard grown as intercrop with lentil is more profitable than sole lentil. The results also suggested that one row of mustard between three rows of lentil was the most suitable intercrop combination for achieving higher economic benefit.

TABLE 5
COST AND RETURN ANALYSIS OF LENTIL-MUSTARD INTERCROPPING SYSTEM DURING RABI SEASON OF 2013-14

Treatment	LER	Average LEY (t/ha)	Average Gross return (Tk)	Average Cost of prod^n(tk)	Average Net return (Tk)	BCR
T_1	1	1.95	91680	78000	13680	1.17
T_2	1	0.71	34080	31000	3080	1.09
T_3	1.65	2.09	100320	81800	18520	1.23
T_4	1.62	2.10	100800	80000	20800	1.26
T_5	1.54	2.23	107040	79200	27840	1.35
T_6	1.35	1.93	92640	78500	14140	1.18
T_7	1.24	1.80	86400	81300	5100	1.06

TABLE 6
COST AND RETURN ANALYSIS OF LENTIL-MUSTARD INTERCROPPING SYSTEM DURING RABI SEASON OF 2014-15

Treatment	LER	LEY (t/ha)	Gross return (Tk)	Cost of prod^n (tk)	Net return (Tk)	BCR
T_1	1	1.99	159200	78000	74800	2.04
T_2	1	0.74	59200	31000	28000	1.90
T_3	1.65	2.08	166400	81800	84600	2.03
T_4	1.62	2.17	173600	80000	93000	2.17
T_5	1.54	2.28	182400	79200	103200	2.30
T_6	1.35	2.01	160800	82000	78800	1.96
T_7	1.24	1.84	147200	82300	64900	1.78

Where, T_1=Sole Lentil, T_3= One row of Lentil+One row of mustard, T_4=One row of mustard in between every two rows of lentil, T_5= One row of mustard in between alternate three rows of lentil, T_6=One row of mustard in between every four rows of lentil and T_7=Two rows of mustard in between every four rows of lentil.

IV. CONCLUSION

From the study it was found that one row of mustard in between every three rows of lentil performed better than other. Compared to conventional monoculture oflentil, mustard-lentil intercropping had significant advantage in yield, economy, land utilization ratio and reducing soil nitrate-N accumulation, as well as better residual effect on the subsequent crop. Intercropping systems could reduce N fertilizer use and increase relative biomass of respected crops as a result of high photosynthetic efficiency of border rows and sufficient nitrate supply during symbiotic period. Noticeably, intercropping advantage was not inherent but began to emerge at legume formation stage. 3L:1M was the best intercropping system in this study, as it had the largest LER and BCR.

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Tracking of Diversity among a Wide Local Collection of Bitter Gourd (*Momordica charantia L.*) Landraces in Bangladesh

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Abstract— *Genetic diversity of twenty bitter gourd genotypes based on ten characters was measured through multivariate analysis. The 20 genotypes fell into five distant clusters. The cluster IV comprised the maximum number (6) of genotypes followed by same in cluster II and cluster III (5). The cluster I and V comprised 3 and 1genotypes respectively. The highest inter-cluster distance (64.53) was observed between the cluster III and V. The lowest inter-cluster distance (7.05) was observed between the cluster II and III. The inter-cluster distances were larger than the intra-cluster distances. The intra-cluster distance in the entire five clusters was more or less low indicating that the genotypes within the same cluster were closely related. Fruit diameter and fruits per plant were the important component characters having higher contribution to the genetic divergence. Path co-efficient analysis revealed that branch per vine, fruits length, and fruit diameter had positive direct effect on fruit yield. Wide genetic diversity was observed in 20 genotypes of bitter gourd, which were grouped into five clusters. The genotypes of clusters III were more diverse from the genotypes of cluster V.Fruit diameter and fruits per plant were found responsible for the maximum diversity. Hybridization between the genotypes of cluster III and cluster V will manifest the wide genetic variability. Considering group distance and the agronomic performance, the inter genotypic crosses between G16 and G1; G16 and G17; G16 and G10; G16 and G4; G16 and G13 might be suitable choice for future hybridization programme.*

Keywords— Diversity, Path co-efficient, Bitter Gourd (Momordica charantia L.), Landraces, Agronomic performance and Hybridization.

I. INTRODUCTION

Bitter gourd *(Momordica charantia* L.), is an important monoecious and cross- pollinated vegetable crop of the family Cucurbitaceae grown in Bangladesh. It is locally known as karala/uchha.It is extensively cultivated throughout the country under two situations *i.e.* rainy season (July to August) and summer season (February to March). According to Chakravatry (1959), bitter gourd is believed to have originated in the tropics of the old world and is widely distributed in China, Malaya, India, tropical Africa and certain other countries. In terms of nutritive value, bitter gourd ranks first among cucurbits, the most important nutritional contribution being vitamins and minerals especially iron, phosphorus and ascorbic acid. Fruit also contains two alkaloids *viz.*, momordicin and cucurbitacin, momordicin is the momordicosidesglycosides of tetracyclic triterpinoides with cucurbitaneskeleton (Chandra Vadana and Subhash Chandra, 1990).Bitter gourd contains a reasonable amount of different nutrients such as proteins, carbohydrates, fats, minerals and vitamins A, B2, and C etc. Rajasekaran and Shanmugavalu (1984), reported very high amount of vit. C (95 mg/100g) and protein (16.5%) found in some Indian bitter gourd variety. The fruits are bitter to taste due to the presence of substance called cucurbitacin. Bitter gourd is also reported to use against diseases like paralysis, indigestion and vomiting pain and diabetes (Mier and Yaniv, 1985). Fruits and other part of bitter gourd are reported to have cooling, stomachic, appetitising, carminative, antipyrietic, antihelminthic, aphrodisiac and vermifuge properties (Blatter *et al.,* 1935). Various medicinal uses with clinical properties of insulin have been isolated from this species (Baldwa *et al.,* 1977). Among the traditional vegetables bitter gourd occupied important position in export trade. The fruits are used as fried, stuffed, dried and pickled (Morton, 1967). However, inspite of its importance, adoptability and export potential, research priority given to this crop is quite meagre especially on genetic improvement. Among the cucurbits, it is considered a prized vegetable because of its high nutritive values especially ascorbic acid and iron (Behera, 2004). A compound known as charatin present in the bitter gourd is used in the treatment of diabetes to lower blood sugar levels (Shetty *et al.,* 2005). During, 2011-2012 bitter gourds were grown over an area of 9311.74 hectares and its annual production was 46000 Mt (BBS, 2012). During 2013, bitter gourds were grown over an area of 24000

acres, it's per acre yield 2177 kg and annual production was 52000 ton. In Bangladesh, vegetable production is not evenly distributed throughout the year and most of the vegetable are produced during winter (Quasem, 2003; BARI, 2006). Hence there is a severe deficiency of vegetables during summer season due to adverse climatic conditions (Chowdhury, 1993; Ali *et al*., 1993 and Rashid, 1999). The bitter gourd production can meet up the crisis. It grows more or less in every areas of Bangladesh. Young shoots and leaves are extensively used as vegetable in the Philippines where the plants are found in the wild in waste places. The juice of the leaves and fruits of bitter gourd has been used as an anthelmintie, and is applied externally for malignant ulcers (Oliver, 1960). According to Ayensu (1984), the leaves are also used traditionally in the treatment of breast cancer. Bitter gourd may contribute to the nutritional shortage of the people of Bangladesh. Particularly, it can provide added proteins, minerals and vitamins to the diet. Although bitter gourd is an important vegetable crop, there is no recommended variety in Bangladesh and very little is known about its improvement practice. The considerable increases in bitter gourd production is no doubt remarkable, but the fact remains that the bitter gourd growers are surrounded with a number of problems, like the pests and diseases, high labour charge etc. Very few research works relating to diversity of bitter gourd have been conducted in Bangladesh. So, intensive research efforts are needed in several areas, particularly, selection of superior genotypes. There are a lot of variabilities among the existing bitter gourd germplasm of Bangladesh. An understanding of the nature and magnitude of the variability among the genetic stocks of bitter gourd is of prime importance for the breeder. A good knowledge of genetic wealth might also help in identifying desirable cultivars for commercial production. Because of its nature of high cross pollination, hardly any genetically pure strain is available to the growers. Among the local cultivated varieties, a wide range of genetic variability exists in this crop which can be exploited for its improvement. The basic key to a breeder is to develop high yielding varieties through selection, either from the genotypes or from the segregates of a crop. Expression of different plant character is controlled by genetic and environmental factors. In a hybridization program knowledge of interrelationship among and between yield and yield components is necessary. Path analysis partitions the components of correlation co-efficient into direct and indirect and visualizes the relationship in more meaningful way (Bhatt, 1973). Estimation of genetic diversity is considered as an important factor, which is also essential prerequisite for hybridization program for developing high yielding variety. Multivariate analysis is a useful tool in quantifying the degree of divergence between biological populations at genotypic level. Based on the information, the present study was undertaken to know the yield potentiality of genotypes and to know the genetic diversity among the genotypes for future hybridization program.

II. MATERIALS AND METHODS

This chapter deals with the major information regarding materials and methods that were used in conducting the experiment. It consists of a short description of locations of the experimental site, characteristics of soil, climate, materials, layout and design of the experiment, land preparation, manuring and fertilizing, transplanting of seedlings, intercultural operations, harvesting, data recording procedure, economic and statistical analysis etc. The research work relating to determine the genetic diversity of bitter gourds was conducted at the A field experiment was conducted with 20 genotypes of bitter gourd (Momordica charantia L.) at the experimental field of Sher-e-Bangla Agricultural University, Dhaka, to study the genetic diversity among the genotypes for yield and yield contributing characters, estimate genetic parameters, association among the characters and their contribution to yield during April 2015 to September 2015. Soil of the experimental site belongs to the general soil type, Shallow Red Brown Terrace Soils under Tejgaon Series. Top soils were clay loam in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. Soil pH ranged from 6.0- 6.6 and had organic matter 0.84%. Experimental area was flat having available irrigation and drainage system and above flood level. Twenty genotypes of bitter gourd were used for the present research work. The purity and germination percentage were leveled as around 100 and 80, respectively. The genetically pure and physically healthy seeds of these genotypes were collected from Plant Siddiq Bazar, Gulistan, Dhaka, Narayanganj local market, Dhaka, Agargaon local market, Agargaon, Dhaka, Kawran bazar, Dhaka. The name and origin of these genotypes are presented in (Table 1). The experiment was laid out Randomized Complete Block Design (RCBD) with three replications. The genotypes were distributed into the every plot of each block of the prepared layout of the experiment. The individual plot was 3 m × 1 m in size. The twenty genotypes of the experiment were assigned at random into plots of each replication. The distance maintained spacing row to row 50 cm and plant to plant 2 m. The distance maintained between two blocks was 1 m. After final land preparation, pits of 50 cm × 50 cm × 45 cm were prepared in each plot with a spacing of a spacing of 3 m × 1 m. Pits were kept open in the sun for 7 days to kill harmful insect and microorganisms. To control field cricket 5 mg Furadan was also mixed with the soils of each pit before making it ready for dibbling. The following doses of manure and fertilizers were applied to the plots for ridge gourd cultivation (Anonymous, 1991). Total cowdung, half of TSP and one third MOP were applied in the field during final land preparation. Remaining TSP and one third MOP and whole gypsum and zinc oxide and one third of urea were applied in pit one week

prior to transplantation. Remaining urea and MoP were applied as top dressing in four installments at 20, 40, 60 and 75 days after transplanting. Several weeding and mulching were done as per requirement. At the very first stage weeding was done for ease of aeration and less competition seedling growth and mulch was provided after an irrigation to prevent crust formation and facilitate good aeration.

At the seedling stage red pumpkin beetle attacked tender leaves and also after the initial stage they attacked plants several times for this Marathon and Ripcord was sprayed in the field. In mature stage fruit fly caused severe damage to the fruit. For protection from fruit fly, MSGT (Mashed Sweet Gourd Trap) and Pheromone baitwas used along with ripcord, sevin powder. Fruits were picked on the basis of horticultural maturity, size, colour and age being determined for the purpose of consumption as the fruit grew rapidly and soon get beyond the marketable stage, frequent picking was done throughout the harvesting period. Data were recorded on Days to first male flowering, Days to first female flowering, Vine length (m), Number of nodes per vine, Branches per vine, Fruit length (cm), Fruit diameter (cm), Number of fruit per plant, Weight per fruit (g), Yield per plant (kg) parameters from the studied plants during the experiment. The details of data recording are given below on individual plant basis.

TABLE 1
NAME AND ORIGIN OF TWENTY BITTER GOURD GENOTYPES USED IN THE PRESENT STUDY

Sl. No.	Genotypes No.	Location
1	G_1	Agargaon local market, Agargaon, Dhaka
2	G_2	Siddiq Bazar, Gulistan, Dhaka
3	G_3	Narayanganj local market
4	G_4	Agargaon local market, Agargaon, Dhaka
5	G_5	Siddiq Bazar, Gulistan, Dhaka
6	G_6	Agargaon local market, Agargaon, Dhaka
7	G_7	Siddiq Bazar, Gulistan, Dhaka,
8	G_8	Siddiq Bazar, Gulistan, Dhaka,
9	G_9	Narayanganj local market
10	G_{10}	Narayanganj local market
11	G_{11}	Kawran bazar,Dhaka
12	G_{12}	Narayanganj local market
13	G_{13}	Agargaon local market, Agargaon, Dhaka
14	G_{14}	Siddiq Bazar, Gulistan, Dhaka,
15	G_{15}	Kawran bazar,Dhaka
16	G_{16}	Agargaon local market, Agargaon, Dhaka
17	G_{17}	Narayanganj local market
18	G_{18}	Siddiq Bazar, Gulistan, Dhaka,
19	G_{19}	Narayanganj local market
20	G_{20}	Kawran bazar,Dhaka

2.1 Statistical analysis

Mean data of the characters were subjected to multivariate analysis. Univariate analysis of the individual character was done for all characters under study using the mean values (Singh and Chaudhury, 1985) and was estimated using MSTAT-C computer programme. Duncan's Multiple Range Test (DMRT) was performed for all the characters to test the differences between the means of the genotypes. Mean, range and co-efficient of variation (CV %) were also estimated using MSTAT-C. Multivariate analysis was done by computer using GENSTAT 5.13 and Microsoft Excel 2007 software through four techniques viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CA) and Canonical Vector Analysis (CVA).

2.2 Path co-efficient analysis

Path co-efficient analysis was done according to the procedure employed by Dewey and Lu (1959) also quoted in Singh and Chaudhary (1985), using simple correlation values. In path analysis, correlation co-efficient is partitioned into direct and

indirect independent variables on the dependent variable. In order to estimate direct & indirect effect of the correlated characters, say x1, x2 and x3 yield y, a set of simultaneous equations (three equations in this example) is required to be formulated as shown below:

ryxl =Pyxl + Pyx2rxlx2+ Pyx3rxlx3

ryx2= Pyxl rxlx2 + Pyx2 + Pyx3r x2x3

ryx3=Pyxlrxlx3 + Pyx2rx2x3 +Pyx3

Where, r's denotes simple correlation co-efficient and P's denote path co-efficient (Unknown). P's in the above equations may be conveniently solved by arranging them in matrix form. Total correlation, say between x1 and y is thus partitioned as follows:

Pyx1 = The direct effect of x1 on y.

Pyx2rx1x2 = The indirect effect of x1 via x2 on y

Pyx3rx1x3 = The indirect effect of x1via x3 on y

After calculating the direct and indirect effect of the characters, residual effect (R) was calculated by using the formula given below (Singh and Chaudhary, 1985):

$$P2RY = 1 - \sum P_{iy} .riy$$

Where, P2RY = (R2); and hence residual effect, R= (P2RY) ½

Piy = Direct effect of the character on yield

riy = Correlation of the character with yield

2.3 Multivariate analysis

The genetic diversity among the genotypes was assessed by Mahalanobis's (1936) general distance (D^2) statistic and its auxiliary analyses. The parents selection in hybridization programme based on Mahalanobis's D^2 statistic is more reliable as requisite knowledge of parents in respect of a mass of characteristics is available prior to crossing. Rao (1952) suggested that the quantification of genetic diversity through biometrical procedures had made it possible to choose genetically diverse parents for a hybridization programme. Multivariate analysis viz. Principal Component analysis, Principal Coordinate analysis, Cluster analysis and Canonical Vector analysis (CVA), which quantify the differences among several quantitative traits, are efficient method of evaluating genetic diversity. These are as follows:

2.4 Principal Component analysis (PCA)

Principal Component analysis, one of the multivariate techniques, is used to examine the inter-relationships among several characters and can be done from the sum of squares and products matrix for the characters. Thus, PCA finds linear combinations of a set variate that maximize the variation contained within them, thereby displaying most of the original variability in a smaller number of dimensions. Therefore, Principles components were computed from the correlation matrix and genotypes scores obtained for first components (which has the property of accounting for maximum variance) and succeeding components with latent roots greater than unity.Contribution of the different morphological characters towards divergence is discussed from the latent vectors of the first two principal components.

2.5 Principal Coordinate analysis (PCO)

Principal Coordinate analysis is equivalent to PCA but it is used to calculate inter unit distances. Through the use of all dimension of p it gives the minimum distance between each pair of the n points using similarity matrix (Digby*et al.*, 1989).

2.6 Cluster analysis (CA)

Cluster analysis divides the genotypes of a data set into some number of mutually exclusive groups. Clustering was done using non-hierarchical classification. In Genstat, the algorithm is used to search for optimal values of chosen criterion proceeds as follows. Starting from some initial classification of the genotypes into required number of groups, the algorithm repeatedly transferred genotypes from one group to another so long as such transfer improved the value of the criterion.

When no further transfer can be found to improve the criterion, the algorithm switches to a second stage which examines the effect of swooping two genotypes of different classes and so on.

2.7 Canonical Vector analysis (CVA)

Canonical vector analysis (CVA) finds linear combination of original variabilities that maximize the ratio of between group to within group variation, thereby giving functions of the original variables that can be used to discriminate between the groups. Thus, in this analysis a series of orthogonal transformations sequentially maximizing of the ratio of among groups to the within group variations. The canonical vector are based upon the roots and vectors of WB, where W is the pooled within groups covariance matrix and B is the among groups covariance matrix.

2.8 Calculation of D^2 values

The Mahalanobis's distance (D^2) values were calculated from transformed uncorrelated means of characters according to Rao (1952), and Singh and Chaudhury (1985). The D^2 values were estimated for all possible combinations between genotypes. In simpler form D^2 statistic is defined by the formula

$$D^2 = \sum_{i}^{x} d_i^2 = \sum_{i}^{x} (Y_i^j - Y_j^k) \quad (j \neq k)$$

Where,

Y = Uncorrelated variable (character) which varies from i = 1 ------to x

x = Number of characters.

Superscript j and k to Y = A pair of any two genotypes.

2.9 Computation of average intra-cluster distances

Average intra-cluster distances were calculated by the following formula as suggested by Singh and Chuadhury (1985).

$$\text{Average intra-cluster distance} = \frac{\sum D_i^2}{n}$$

Where,

D_i^2 = the sum of distances between all possible combinations (n) of genotypes included in a cluster

n= Number of all possible combinations between the populationsin cluster

2.10 Computation of average inter-cluster distances

Average inter-cluster distances were calculated by the following formula as suggested by Singh and Chuadhury (1985).

$$\text{Average inter-cluster distance} = \frac{\sum D_{ij}^2}{n_i \times n_j}$$

Where,

$\sum D_{ij}^2$ = The sum of distances between all possible

Combinations of the populations in cluster i and j

n_i= Number of populations in cluster i

n_j= Number of populations in cluster j

2.11 Cluster diagram

Using the values of intra and inter-cluster distances ($D = \sqrt{D^2}$), a cluster diagram was drawn as suggested by Singh and Chuadhury (1985). It gives a brief idea of the pattern of diversity among the genotypes included in a cluster.

III. RESULT AND DISCUSSION

This chapter comprises the presentation and discussion of the findings obtained from the study. The data pertaining to 20 bitter gourd genotypes as well as yield and its contributing characters were computed and statistically analyzed and the results thus obtained are discussed below under the following heads:

3.1 Analysis of variance

The analysis of variance indicated significantly higher amount of variability among the genotypes for all the characters studied *viz*., vine length, branch per vine, nodes per vine, days to first male flowering, days to first female flowering, fruit length, fruit diameter, fruit weight, fruits per plant and fruits yield per plant (Table 2). The variation due to replication was non-significant for all the characters studied.

3.2 Path coefficient analysis

Though correlation analysis indicates the association pattern of components traits with yield, they simply represent the overall influence of a particular trait on yield rather than providing cause and effect relationship. The technique of path coefficient analysis developed by Wright (1921) and demonstrated by Dewey and Lu (1959), facilitates the portioning of correlation coefficients into direct and indirect contribution of various characters on yield. It is standardized partial regression coefficient analysis. As such, it measures the direct influence of one variable upon other. Such information would be of great value in enabling the breeder to specifically identify the important component traits of yield and utilize the genetic stock for improvement in a planned way.

In path coefficient analysis the direct effect of a trait on fruit yield per plant and its indirect effect through other characters were computed and the results are presented in Table 3.

3.3 Direct effect

Three out of nine characters had positive direct effect on fruit yield per plant. The characters which had positive direct effect were branches per vine (0.88), fruit length (3.15) and fruit diameter (1.62). However, character *viz.,* vine length (-0.26), nodes per vine (-1.25), days to first male flowering (-0.03), days to first female flowering (-0.54), fruit weight (-3.00) and fruits per plant (-0.13) had negative direct effect on fruit yield (Table 3). Path coefficient analysis revealed that fruit yield per plant was directly influenced by branches per vine, fruit length and fruit diameter. Hence, selection for any of these independent traits leads to improving the genotypes for fruit yield per plant.

3.4 Indirect effects

Days to fist male flowering had negative indirect effect through vine length (-0.01), branches per vine (-0.21), nodes per vine (-0.51), days to first female flowering (-0.31) and fruit diameter (-0.19) (Table 3). However, its indirect effects through fruit length (0.77), fruit weight (0.18) and fruits per plant (0.08). The effect of days to first female flowering to fruit yield per plant through fruit diameter (0.25), fruit weight (0.79) and fruits per plant (0.05) was remarkable, its contribution through other traits was low. Vine length influenced the fruit yield per plant indirectly through fruit length (0.43) and fruit diameter (0.51) (Table 3). The indirect and positive effect on fruit yield per plant was exhibited by nodes per vine via fruit length (0.62), fruit diameter (0.14), fruit weight (0.18) and fruits per plant (0.03) Whereas, through other traits it had also negative indirect effects. Branches per vine showed positive indirect effect to fruit yield per plant via vine length (0.01), nods per vine (0.19), days to first male flowering (0.01), days to first female flowering (0.09) (Table 3). It had a negative indirect effect through fruit length (-0.47) and fruit diameter (-0.29).Fruit length showed indirect effect on fruit yield per plant had positive through days to first female flowering (0.08) (Table 3).Fruits per plant had positive indirect effect through branches per vine (0.21), nodes per vine (0.31), days to first female flowering (0.20) and fruit diameter (0.26) to fruit yield per plant (Table 3). This trait showed negative indirect effect via fruit length (-0.11) and fruit weight (-0.37) (Table 3).Fruit weight showed indirect positive effects on fruit yield per plant by nodes per vine (0.07), days to first female flowering (0.14), fruit length (2.60) and fruit diameter (0.36) (Table 3). It showed indirect negative effect on fruit yield per plant through vine length (-0.05) and fruits per plant (-0.02) (Table 3).From the present path analysis study in bitter gourd, it may be concluded that improvement in fruit yield per plant could be brought by selection for component characters like branches per vine, fruit length, fruits per plant and fruit diameter.

TABLE 2

ANALYSIS OF VARIANCE OF DIFFERENT CHARACTERS IN BITTER GOURD

Source	Df	Mean sum of square									
		DFMF	DFFF	VL	NPV	BPV	FL	FD	FPP	FW	FYP
Rep	2	44.60	56.12	0.01	44.82	0.47	0.91	1.52	64.12	538.02	0.970
Treatment	19	19.55**	28.89**	0.18**	16.71**	43.42**	27.49**	2.85**	21.39**	1731.10**	0.361**
Error	38	6.39	7.71	0.01	2.76	2.13	2.72	1.36	2.77	121.51	0.058

***** Correlation is significant at the 0.01 level.***

DFMF = Days to first male flowering, DFFF = Days to first female flowering, VL = Vine length (M), BPV = Branches per vine, NPV = Nodes per vine, FL = Fruit length (cm), FD = Fruit diameter (cm), FPP = Fruits per plant, FW = Fruit weight (g), FYP = Fruits yield per plant (Kg).

TABLE 3

PATH COEFFICIENT ANALYSIS SHOWING DIRECT AND INDIRECT EFFECTS OF DIFFERENT CHARACTERS ON YIELD OF BITTER GOURD

	Direct effect	Indirect effect									Genotypic correlation with yield
		DFMF	DFFF	VL	NPV	BPV	FL	FD	FPP	FW	
DFMF	-0.03	-	-0.31	-0.01	-0.51	-0.21	0.77	-0.19	0.08	0.18	-0.232
DFFF	-0.54	-0.02	-	-0.04	-0.42	-0.15	-0.45	0.25	0.05	0.79	-0.539**
VL	-0.26	0.00	-0.08	-	-0.02	-0.02	0.43	0.51	-0.02	-0.51	0.026
NPV	-1.25	-0.01	-0.18	0.00	-	-0.14	0.62	0.14	0.03	0.18	-0.620**
BPV	0.88	0.01	0.09	0.01	0.19	-	-0.47	-0.29	-0.03	0.00	0.378**
FL	3.15	-0.01	0.08	-0.04	-0.24	-0.13	-	-0.38	0.00	-2.48	-0.062
FD	1.62	0.00	-0.08	-0.08	-0.10	-0.16	-0.75	-	-0.02	-0.66	-0.231
FPP	-0.13	0.02	0.20	-0.04	0.31	0.21	-0.11	0.26	-	-0.37	0.346**
FW	-3.00	0.00	0.14	-0.05	0.07	0.00	2.60	0.36	-0.02	-	0.117

Residual effect: **0.342**

** = Significant at 1%, * = Significant at 5%.

DFMF = Days to first male flowering, DFFF = Days to first female flowering, VL = Vine length (M), BPV = Branches per vine, NPV = Nodes per vine, FL = Fruit length (cm), FD = Fruit diameter(cm), FPP = Fruits per plant, FW = Fruit weight (g), FYP = Fruits yield per plant (Kg).

TABLE 4

DISTRIBUTION OF TWENTY GENOTYPES IN DIFFERENT CLUSTERS

Cluster no.	No. of Genotypes	No. of populations	Name of genotypes
I	3, 7, 12	3	G3, G7, G12
II	8, 9, 14, 15, 20	5	G8, G9, G14, G15, G20
III	1, 4, 10, 13, 17	5	G1, G4, G10, G13, G17
IV	2, 5, 6, 11, 18, 19	6	G2, G5, G6, G11, G18, G19
V	16	1	G16
Total	20		

3.5 Genetic diversity

The knowledge of available genetic diversity is an important factor for any heritable improvement and its nature and degree is useful for selecting desirable parents from a germplasm for the successful breeding programme. There is still much scope for improving of genetic architecture desirable for hybrid through heterosis breeding. Its magnitude in desirable direction is preferable. The success of hybridization depends upon the selection of suitable parental genotypes and performance of their cross combinations.

3.6 Nonhierarchical clustering

With the application of covariance matrix for nonhierarchical clustering, 20 Bitter gourd genotypes were grouped into five different clusters. It is stated that the highest 30% genotypes were included in cluster IV and it was followed by 25% in both cluster II and III, 15% genotypes in cluster I, and the remaining 5% genotypes were in cluster V. The composition of clusters with different genotypes is presented in (Table 4).Cluster IV had the maximum 6 genotypes (G2, G5, G6, G11, G18, G19) followed by cluster II which had 5 genotypes (G8, G9, G14, G15, G20), cluster III also had 5 genotypes (G1, G4, G10, G13, G17) and cluster I had 3 genotypes (G3, G7, G12). Cluster V comprised with one genotype (G16) (Table 4).

3.7 Principal component analysis (PCA)

Eigen values of principal component axis, percent of total variation and cumulative variation accounted for them obtained from principal component analysis are presented in (Table 5). The results showed that the first principal axis, vile length (m) largely accounted for the variation among the genotypes which alone contributed 24.96% of the total variation among the genotypes. The first seven characters of the principal component axes with eigen values above unity accounted for 91.24% of the total variation among the ten characters. The rest three characters contributed remaining 8.76% of total variation. Based on principal component scores I and II obtained from the principal component analysis, a two-dimensional scatter diagram (Z_1-Z_2) using component score 1 as X axis and component score 2 as Y axis was constructed, which has been presented in (Figure 1 and Table 11).

TABLE 5

EIGEN VALUES AND PERCENTAGE OF VARIATION FOR CORRESPONDING 10 COMPONENT CHARACTERS IN 20 GENOTYPES OF BITTER GOURD

Principal component axes	Eigen values	Percent variation	Cumulative % of Percent variation
I	2.496	24.96	24.96
II	1.945	19.45	44.41
III	1.374	13.74	58.15
IV	0.995	9.95	68.10
V	0.927	9.27	77.37
VI	0.770	7.70	85.07
VII	0.617	6.17	91.24
VIII	0.456	4.56	95.80
IX	0.380	3.80	99.60
X	0.041	0.40	100.00

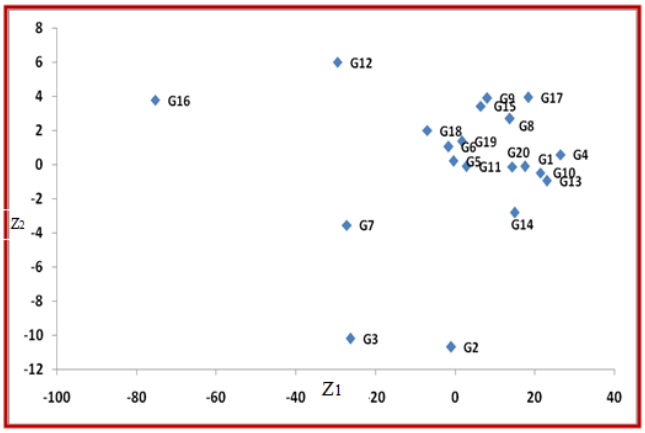

FIGURE 1. SCATTER PATTERN OF BITTER GOURD GENOTYPES OF BASED ON THEIR PRINCIPAL COMPONENT SCORES

3.8 Inter cluster distance

The inter cluster D^2 values are given in (Table 6) and the nearest and farthest cluster from each cluster based on D^2 value is given in (Table 7). The inter cluster D^2 values were maximum (64.53) between the cluster III and cluster V, followed by II and V (57.96) and IV and V (48.98). The higher inter-cluster distances between these clusters indicate to obtain wide spectrum variability of population. However, the highest inter cluster distance was observed between clusters III and V indicated the genotypes in these clusters were diverse than those clusters. Cluster V was the most diverse as many other clusters showed the maximum inter cluster distance with it (Table 7). The minimum distance observed between clusters II and III (7.05) indicated close relationship among the genotypes included.

3.9 Intra cluster distance

The intra cluster D^2 values were given in (Table 6). The intra cluster distance was observed in the clusters I, II, III and IV. The intra cluster distance was higher in cluster I (0.330) followed by cluster IV (0.240) and The lowest in cluster II (0.067). No intra cluster distance was observed for cluster V because of one genotype included in this cluster. The intra cluster distances in all the five clusters were lower than the inter cluster distances and which indicated that genotypes within the same cluster were closely related. The inter cluster distances were larger than the intra cluster distances which indicated wider genetic diversity among the genotypes of different groups.

3.10 Cluster diagram

The positions of the genotypes in the scatter diagram were apparently distributed into five groups, which indicated that considerable diversity existed among the genotypes (Figure 2).

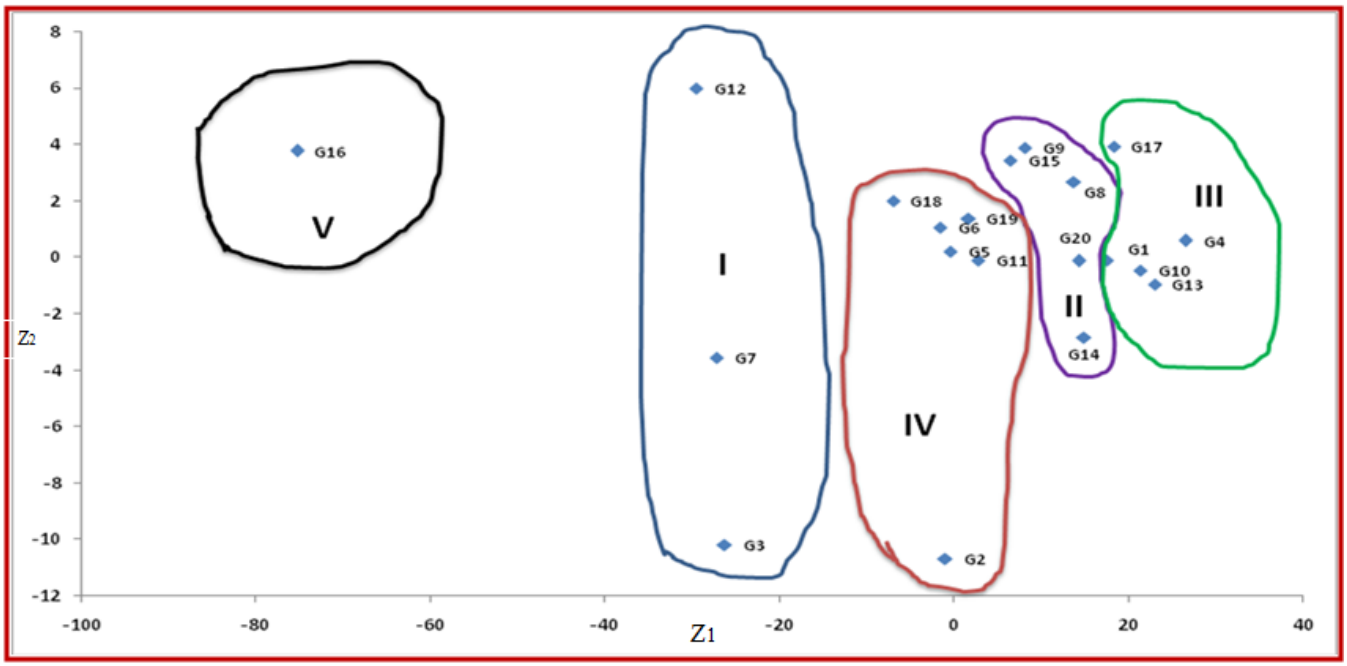

FIGURE 2. SCATTER DIAGRAM OF BITTER GOURD GENOTYPES OF BASED ON THEIR PRINCIPAL COMPONENT SCORE

TABLE 6
INTRA (BOLD) AND INTER CLUSTER DISTANCES (D^2) FOR 20 GENOTYPES OF BITTER GOURD

Cluster	I	II	III	IV	V
I	**0.330**	29.82	36.58	20.86	28.61
II		**0.067**	7.05	9.72	57.96
III			**0.165**	16.44	64.53
IV				**0.240**	48.98
V					**0.00**

TABLE 7
THE NEAREST AND FARTHEST CLUSTERS FROM EACH CLUSTER BETWEEN D^2 VALUES OF BITTER GOURD

Sl No.	Cluster	Nearest Cluster with D^2 values	Farthest Cluster with D^2 values
1	**I**	**IV (**20.86)	**III (**36.58)
2	**II**	**III (**7.05)	**V (**57.96)
3	**III**	**II (**7.05)	**V(**64.53)
4	**IV**	**II (**9.72)	**V (**48.98)
5	**V**	**I (**28.61)	**III (**64.53)

TABLE 8
CLUSTER MEAN VALUES OF 10 DIFFERENT CHARACTERS OF 20 GENOTYPES OF BITTER GOURD

Characters	I	II	III	IV	V
Vine length (m)	4.52	4.32	4.20	4.17	4.25
Branches per vine	36.00	38.53	40.07	35.39	43.00
Nodes per vine	84.22	83.67	83.07	81.39	81.00
Days to first male flowering	55.44	53.00	54.33	53.89	51.67
Days to first female flowering	60.78	61.33	63.60	59.89	61.33
Fruit length (cm)	24.88	18.55	17.96	19.60	25.90
Fruit diameter (cm)	13.14	13.19	12.81	13.63	13.50
Fruit weight (g)	135.55	96.80	87.07	109.17	183.33
Fruits per plant	22.89	23.20	20.80	21.33	22.00
Fruit yield per plant (Kg)	2.18	2.33	2.12	2.39	2.47

3.11 Cluster mean analysis

The cluster means of 10 different characters (Table 8) were compared and indicated considerable differences between clusters for all the characters studied. The maximum vine length was observed in cluster I (4.52), whereas the minimum vine length was observed in cluster IV (4.17). The maximum (43.00) and the minimum (35.39) branches per vine were observed in cluster V and IV respectively. Genotypes in cluster V showed the lowest nodes per vine (81.00) and that in cluster I had the highest mean (84.22) nodes per vine. The maximum (55.44) and the minimum (51.67) days to first male flowering were observed in cluster I and V respectively. The maximum days to first female flowering were observed in cluster III (63.60), whereas the minimum days to first flowering were observed in cluster IV (59.89). Cluster V had the maximum fruit length (25.90), cluster III had the minimum fruit length (17.96). The maximum fruit diameter (13.63) was observed in the cluster IV, whereas the minimum fruit diameter (12.81) was observed in cluster III. Fruit weight was the highest in cluster V with a mean value of (183.33) and it was least in genotypes belongs to the cluster III (87.07). The highest fruits per plant were recorded by the cluster II (23.20) while cluster III (20.80) showed the least fruits per plant. The maximum fruit yield per plant was observed in cluster V (2.47), whereas the minimum fruit yield per plant was observed in cluster III (2.12).

3.12 Contribution of characters towards divergence

Contribution of characters towards the divergence obtained from canonical varieties analysis is presented in (Table 9). The character, which gave high absolute magnitude for vector 1, was considered to be responsible for primary differentiation. Likewise, the characters, which gave higher absolute magnitude for vector 2 was considered to be responsible for secondary differentiation. If the same character given equal magnitude for both the vectors than the character was considered responsible for primary as well as secondary differentiation.

TABLE 9

RELATIVE CONTRIBUTIONS OF THE THIRTEEN CHARACTERS OF 20 VARIETIES TO THE TOTAL DIVERGENCE

Characters	Principal Component	
	Vector-1	Vector-2
Vine length (m)	-4.1397	-1.4697
Branch per vine	0.2165	-0.3741
Nodes per vine	0.3173	-0.1575
Days to first male flowering	-0.0466	0.1198
Days to first female flowering	-0.0009	-0.3257
Fruit length (cm)	-0.8297	0.3994
Fruit diameter(cm)	0.4920	1.3151
Fruit weight (g)	-0.6014	-0.0662
Fruits per plant	0.0992	0.0280
Fruits yield per plant (Kg)	-1.0496	2.5485

TABLE 10

SALIENT FEATURES OF GENOTYPES IN FIVE DIFFERENT CLUSTERS

Cluster	Salient features
I	Long vine, More nodes per vine
II	More fruits per plan, Medium nodes
III	Moderate branches per vine, Late female flowering
IV	Early female flowering, Higher fruit diameter
V	Highest branches per vine, Early male flowering, Highest fruit length, More fruit weight, Highest fruit yield per plant

TABLE 11

PRINCIPAL COMPONENT SCORE 1 & 2.

Genotypes	Z_1	Z_2
1	17.506	-0.107
2	-1.006	-10.665
3	-26.288	-10.196
4	26.485	0.596
5	-0.425	0.208
6	-1.662	1.056
7	-27.2	-3.541
8	13.715	2.692
9	8.066	3.889
10	21.343	-0.472
11	2.769	-0.101
12	-29.551	6.004
13	23.009	-0.938
14	14.871	-2.822
15	6.45	3.434
16	-75.309	3.78
17	18.3	3.937
18	-7.017	2.002
19	1.645	1.371
20	14.299	-0.128

In vector (Z_1) obtained from PCA, the important characters responsible for genetic divergence in the axis of differentiation were days to first male flowering (0.119), fruit length (0.3994), fruit diameter (1.3151), fruits per plant (0.0280) and fruit yield per plant (2.5485) were important because all these characters had positive signs (Table 9).

On the other hand, vine length, days to first male flowering, days to first female flowering, branches per vine, nodes per vine, days to first female flowering, possessed the negative sign in the first axis of differentiation and vine length, branches per vine, nodes per vine, days to first female flowering and fruit weight possessed negative signs in the second axis of differentiation that means it had minor role in the genetic diverse. Fruit diameter and fruits per plant had positive signs in both the vectors, which indicated they were the important component characters having higher contribution to the genetic divergence among the materials studied.

3.13 Salient feature cluster`s genotype

The genotypes of cluster I was the best in terms of long vine and more nodes per vine (Table 10). The genotypes of cluster II produced more fruits per plant and medium nodes per vine. The genotype of cluster III possessed moderate branches per vine and late female flowering. The genotypes of cluster IV produced early female flowering and the highest fruit diameter and the cluster V possessed the highest branches per vine, early male flowering, the highest fruit length, more fruit weight, the highest fruit yield per plant**.**

IV. CONCLUSION

Path co-efficient analysis revealed that branch per vine, fruits length, and fruit diameter had positive direct effect on fruit yield. Wide genetic diversity was observed in 20 genotypes of bitter gourd, which were grouped into five clusters. The genotypes of clusters III were more diverse from the genotypes of cluster V.Fruit diameter and fruits per plant were found responsible for the maximum diversity. Hybridization between the genotypes of cluster III and cluster V will manifest the maximum heterosis and create wide genetic variability. The highest heterosis would be manifest in cross combination involving the genotypes belonging to divergent clusters. Considering group distance and the agronomic performance, the inter genotypic crosses between G16 and G1; G16 and G17; G16 and G10; G16 and G4; G16 and G13 might be suitable choice for future hybridization programme.

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Floristic Structure of Yukarisevindikli Natural Pasture in Tekirdag, Turkey

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Abstract— *This research was conducted in Yukarisevindikli village natural pasture in Tekirdag province at Trakya (Thrace) region in Turkey. The objective of this study was to determine the relation between plant species composition and different management techniques of Yukarisevindikli natural pasture. Measurements on pasture were made at 3 different management system (grazed, abondened, mowed) units. Some ecological indicators were investigated such as frequency families, lifeforms, life spam and phytogeographical regions. The most widely spread species on grazed and abondened units were scented grass (Chrysopogon gryllus). Lolium perenne and Trifolium repens were determined common in mowed pasture units. Hemicryptophytes were dominant in the investigated area, followed by therophytes, chamaphytes and geophytes.*

Keywords— Pasture, biodiversity, land use, vegetation.

I. INTRODUCTION

Vegetation ecology, the study of the plant cover and its relationships with the environment, also called synecology, is a complex scientific undertaking, both regarding the overwhelming variation of its object of study in space and time, and its intricate interactions with abiotic and biotic factors (Van der Maarel, 2005). Vegetation is a plant community which is in a land portion and it is affected by environmental factors, affecting each other mutually. Nevertheless, hosting many plant species, soil organisms, and wild animals is among the ecological functions of pastures. Understanding the biological diversity of natural pastures can lead to improvement of pastures and optimal use (Tuna, 2010). Livestock products provide the major economic return from most range and pasture lands and compared with harvested or purchased feeds, pastures and pasture provide a relatively inexpensive and energy-efficient feed source for livestock (Valentine, 1990).This natural pasture are rich in terms of biological diversity. The many vegetation types and species diversity often found in natural and seminatural pastures are an important part of biodiversity. Floristic studies are fundamental for the applied sciences such as pasture management and conservation (Jankju et al.2011). There are about 12,000 flowering plant taxa in Turkey's flora and approximately 4,000 of them are endemic (Davis, 1985; Kaya, 2014).This number increases every day with the identification of new species Turkey. This diversity is presently threatened by intensive agriculture. Biodiversity conservation will require management to improve the condition and cover the native vegetation within the productive agricultural landscape (McIntyre and Hobbs 1999, 2001, Fischer *et al.* 2005, Vesk and Mac Nally2006). Phytosociological studies are essential for protecting the natural plant communities and biodiversity as well as understanding the changes experienced in the past and continuing on into the future (Sağlam , 2013). Call and Roundy (1991) stated that ''A more mechanistic research approach is needed to better understand factors governing germination, seedling establishment, and plant community development in natural and synthetic systems to guide revegetation toward biological diversity.'' The objective of this study was to determine the relation between plant species composition and different management techniques of Yukarisevindikli natural pasture.

II. MATERIAL AND METHODS

The study area is situated in Yukarisevindikli village of Tekirdag city. Latitude 41°23' 47 N Longitude 27°34'54 E of Yukarisevindikli village, Tekirdag (Europe Part) in Turkey (Anonymous 2016).This zone belongs to the A1(E) grid-square in flora of Turkey according to Davis (1985) grid system. In this study, the vegetation analysis was performed according to traditional Braun-Blanquet's «floristic unit system». The relieves were determined by according to «minimal area» method which was 16 m^2 . Measurements on pasture were made at 3 different units. We determined differences found in the species composition between grazed, abondened, mowed sites. Measurements were made from 1May to 15 July 2013. 22 study sites

were selected for vegetation analyses . These sites were grazed, abondened and mowed. Presence/absence data of all vascular plant species were recorded in the sites. Each sites consisted of a 16 m^2 plot. The lists of species, floristic characteristics such as life forms and life span, phytogeograhical region levels of these species, their families, are recorded. The plants of which herbariums and phytogeograhical region are made by collecting out of research area are determined according to the principles which Davis (1985) pointed out in Flora of Turkey. The taxa can also be classified according to their life forms Raunkiaer (1934), Raunkiaer's (1934) classification, five major classes, arranged according to increased protection of the renewing buds: phanerophytes, chamaephytes, hemicryptophytes, cryptophytes, and therophytes.

Percentages of frequency are separated as 5 frequency classification as in the following. In this process, the frequency data were transformed using a five point scale:

I = 1-20%, II = 21-40%, III = 41-60%, IV=61-80%, V = 81-100%.

Frequency = (number of quadrate a species occurs in / total number of quadrates analysed) x100%,

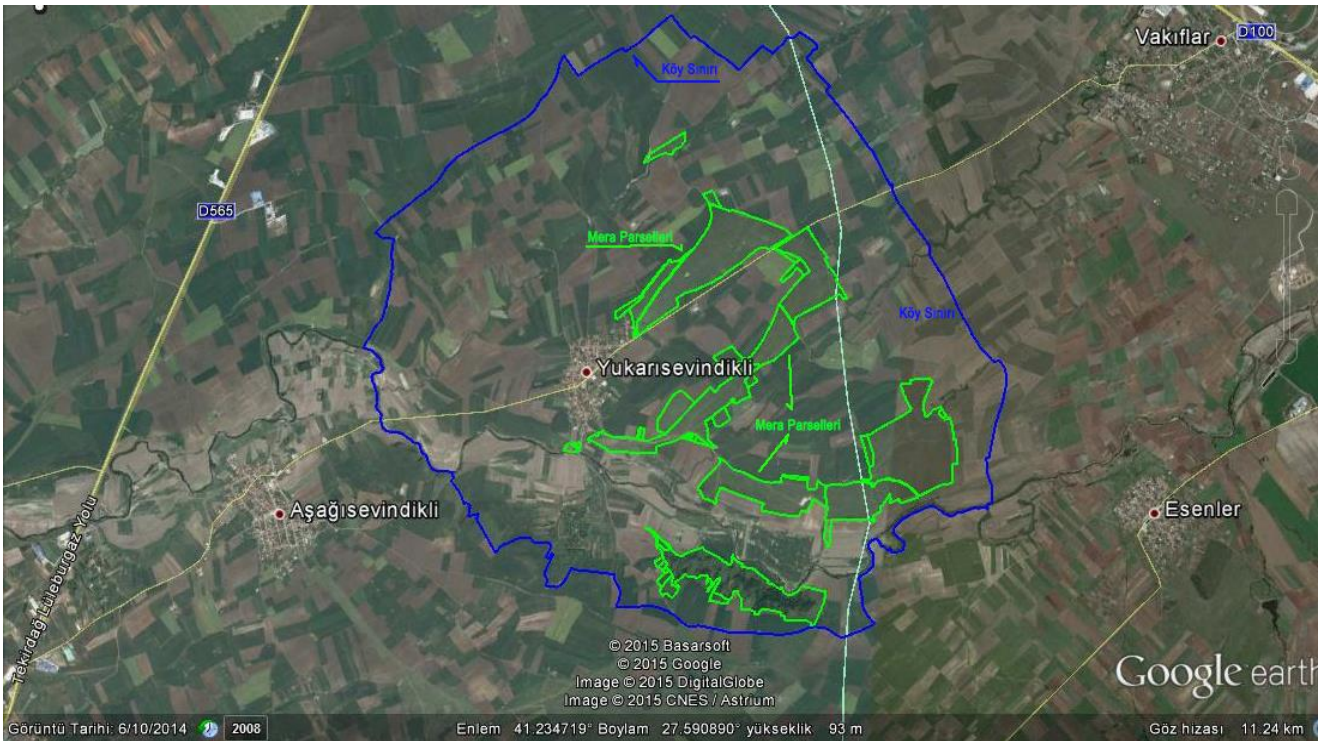

FIG. 1: Google earth image of Yukarisevindikli pasture

Climatic data representing the study area were provided by Tekirdag meteorological stations. Long-term total precipitation and annual mean temperature were 585 mm and 14°C, respectively. Tekirdag examined in the gross of Trakya (Thrace) lands is extremely young geologically. The dominant land types are limeless brown lands and grumusol in Trakya (Thrace). Following these two fundamental land types, rendzina and brown forests and potzolic lands cover large areas. There is also azonal land types except for these lands included in zonal and intrazonal groups in the region. These are usually new alluviums and hydromorphic saltya alluviums under the name of alluvium.

III. RESULTS AND DISCUSSIONS

3.1 Frequency of Pasture Species

Livestock grazing has a considerable effect on community structure and floristic composition (Milchunas and Lauenroth, 1993; Bullock et al. 1995; Dengler et al. 2014). Biodiversity can alter due to grazing, mowed or abondened of pasture. Vandvik and Birks, (2002) stated that land use has strongly influenced vegetation cover and distribution. The change in species composition was related to the change in structure from open to semi-open grasslands under active grazing to close in abandoned sites (Vassilev et al.2011).

TABLE 1
FREQUENCY RATIO OF DOMINANT SPECIES IN GRAZED, MOWED AND ABONDENED PASTURE UNITS

Grazed Pasture	F%	Mowed Pasture	F%	Abondened	F%
Chrysopogon gryllus	100	*Trifolium repens*	100	*Chrysopogon gryllus*	87.5
Dactylis glomerata	100	*Lolium perenne*	100	*Dactylis glomerata*	75
Aegilops triuncialis	87.5	*Vicia villosa*	87.5	*Sanguisorba minor*	63.5
Vulpia ciliata	87.5	*Carex flacca*	87.5	*Trifolium arvense*	63.5
Avena fatua	87.5	*Trifolium campestre*	87.5	*Achillea millefolium*	63.5
Festuca ovina	87.5	*Chrysopogon gryllus*	75	*Bromus tectorum*	50
Thymus longicaulis	87.5	*Hordeum murinum*	75	*Rumex crispus*	50
Eryngium campestre	75	*Achillea millefolium*	75	*Trifolium campestre*	50
Aegilops geniculata	75	*Poa trivialis*	63.5	*Anthemis tomentosa*	50
Bromus tectorum	75	*Medicago minima*	63.5	*Hypericum thasium*	37.5
Trifolium campestre	75	*Plantago lanceolata*	63.5	*Poa trivialis*	37.5
Cirsium laniflorum	63.5	*Hordeum bulbosum*	63.5	*Medicago minima*	37.5
Rumex acetosella	63.5	*Trifolium arvense*	63.5	*Koeleria lobata*	37.5
Convolvulus elegantissimus	63.5	*Lotus corniculatus*	63.5	*Plantago lanceolata*	37.5
Centaurea cyanus	63.5	*Cynosourus echinatus*	63.5	*Lolium perenne*	37.5
Poa bulbosa	50	*Ornithogalum montanum*	63.5	*Festuca ovina*	37.5
Hypericum thasium	50	*Cynodon dactylon*	63.5	*Hordeum bulbosum*	37.5
Cynodon dactylon	50	*Rumex acetosella*	50	*Trifolium subterraneum*	37.5
Poa trivialis	50	*Gastridium phleoides*	50	*Convolvulus elegantissimus*	37.5
Medicago minima	37.5	*Trifolium subterraneum*	50	*Cynodon dactylon*	37.5
Koeleria lobata	37.5	*Aira caryophyllea*	50	*Vicia villosa*	37.5
Vicia sativa	37.5	*Sinapis arvensis*	50	*Anchusa azurea*	25
Bromus rubens	37.5	*Convolvulus elegantissimus*	50	*Pallenis spinosa*	25
Medicago falcata	37.5	*Trifolium nigrescens*	50	*Avena fatua*	25
Geranium dissectum	37.5	*Dactylis glomerata*	37.5	*Thymus longicailus*	25

F: Frequency

In this research,the high percentage of frequency recorded for *Chrysopogon gryllus, Dactylis glomerata, Vulpia ciliata, Festuca ovina, Avena fatua, Thymus longicaulis, Aegilops triuncialis* indicated a high adaptation of these species to livestock grazing. Moreover, the more common species *were determined Lolium perenne, Trifolium repens, Chrysopogon gryllus, Carex flacca, Trifolium campestre* in vegetation structure of mowed pasture while the more common species were *Chrysopogon gryllus, Dactylis glomerata, Sanguisorba minor,* in vegetation structure of abondened pasture (Table 1).Specially, the most widely spread species on Tekirdag pasture was scented grass *(Chrysopogon gryllus*) (Uluocak, 1974; Davis, 1985; Tuna et al. 2011) According to previous research Adams et al. (1986), grasses are usually dominant in pastures all over the world. Indeed, it was determined that *Chrysopogon gryllus* among these common species are found in grazed dry pasture sites. *Chrysopogon gryllus,* grows on warm, dry, illuminated, sandy grassy slopes and hills as well as on dry pasture land (Djurdjevıć et al.2005, Dajıć Stevanovıć et al. 2008).

3.2 Families of Pasture Species

In the protected pasture sites, the three families which have the largest number of species are; Poaceae (35.11%), Fabaceae (19.84%), Asteraceae (16.79%) and other families (28.26%) (Figure 2).According to Adams et al. (1986), grasses are dominant in many pastures all over the world. Erik and Tarıkahya, 2004 .said that the first three families according to number of species in Turkey are *Asteraceae*, *Fabaceae* and *Lamiaceae* which agreed with this research. Fabaceae, Poaceae and Asteraceae members is characterised by step vegetation(Atamov et al. 2007). The fact that therophytes whichconsist of an important part of these three families are found abundant in Mediterranean region makes these families to be on the first line. In this research, Poaceae was the largest family, being the fourth largest family of the flora in Turkey and having wide

toleration limits and involving large genera that contain many species. Lauenroth (1979) said that fundamental family of pasture vegetations are Poaceae and they are common especially in the districts in which the total rainfall is between 250-1000 mm and a lot of pastures around the world has a large number of Poaceae species and, thus, they are called as «Grassland». Grasses formed dense communities in large areas of this pasture as in the local pastures, thereby increasing the area covered by vegetation. Similar results were obtained from the studies conducted in this type of pastures that are mainly made up of graminae. However, based on the results of one recent study carried out in Turkey, plant species of other families were dominant in areas of pasture that were grazed excessively, although grasses were dominant in protected areas of pasture (Vassilev et al.2011). Asterecae is in the third family, and related to being the largest family of the flora of Turkey, having many family members and greater ecological toleration and breaking up seeds easily (Cansaran 2002). Asteraceae has been adapted to these arid and semiarid conditions with a wide diversity (Mood 2008). Abondened pasture units are secondary succesion. Therefore, Asterecea proportion can be higher than others.

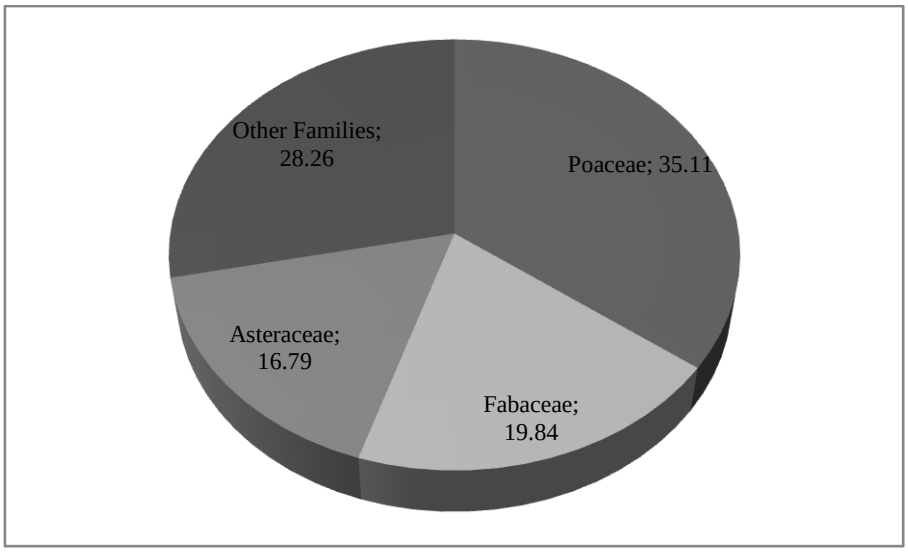

FIG. 2: Distribution of families in abondened pasture units (%)

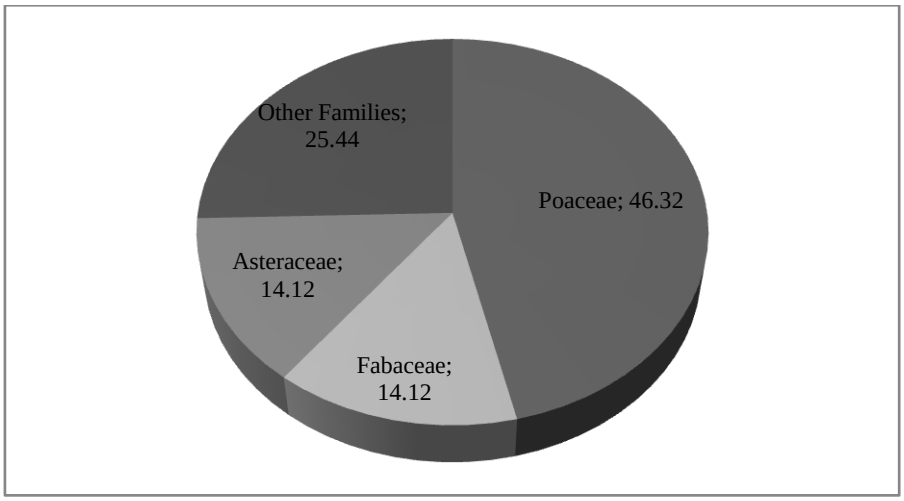

FIG. 3: Distribution of families in grazed pasture units (%)

In the grazed sites, the three families which have the largest number of species are; Poaceae (46.32%), Fabaceae (14.12%), Asteraceae (14.12%) and other families (25.44%) (Figure 3).The rate of the species belonging to the Poaceae family evaluated as productive plants of pastures was high in the research area indicator in pasture, as species may vary significantly in their acceptability to grazing herbivores, not only due to differences in palatability, but also due to phonological differences. In addition to, Fabaceae proportion lowers than other units. It has decreased due to grazing. Hence sheeps specially prefer legumes.

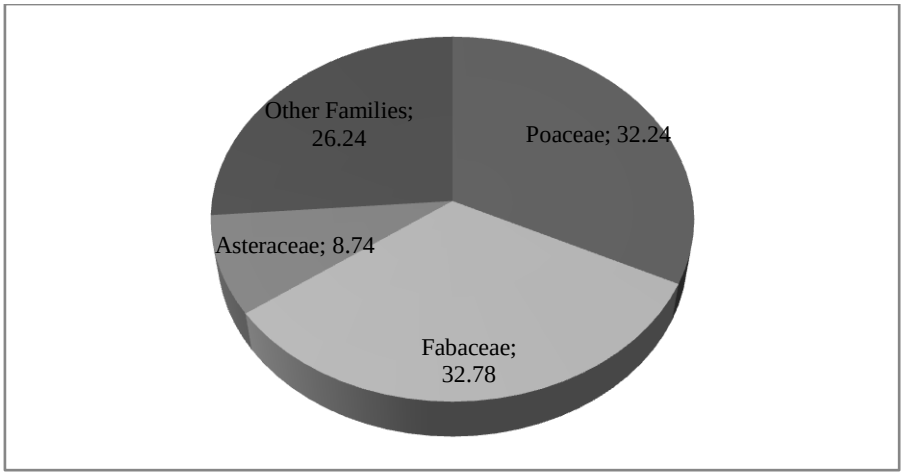

FIG. 4: Distribution of families in mowed pasture units (%)

In the mowed sites, the three families which have the largest number of species are; Poaceae (32.24%), Fabaceae (32.78%), Asteraceae (8.74%) and other families (26.24%) (Figure 4). Fabaceae family's proportion was determined higher than other pasture units. Fabaceae in grasslands are often therophytes with a mesomorph leaf anatomy and persistent seed bank (Dupre and Diekmann 2001). Lok and Fraga (2008) stated that Fabaceae makes that the combined action of sunshine and wind be lower on the soil, favoring higher conservation of humidity and, from the ecological point of view, improving the grasslands conditions. In my previous study, 27.7, 31.0, and 41.3 % distribution were obtained according to the Poaceae, Fabaceae, and other family species in Trakya region pastures (Tuna 2000).

3.3 Life Form of Pasture Species

A life-form is characterized by the adaptation of the plants to certain ecological conditions (Mera et al. 1999). Raunkiaer (1934), explains the form of life, organs keeping generations of plants namely sprouts and buds as a protection kind and situation in the unfavourable season (in cold and dry conditions) and in the term of rest of vegetation. Plant life form composition was consistent with climate, floristic composition, and habitats (Wang 2002 a,b). Life forms reflect a particular strategy of resource use, and their diversity is significantly correlated with climatic heterogeneity (Cowling et al.1994).Diaz et al. (2007) stated that plant functional type and response rules need to be defined for each different climatic context and grazing history.

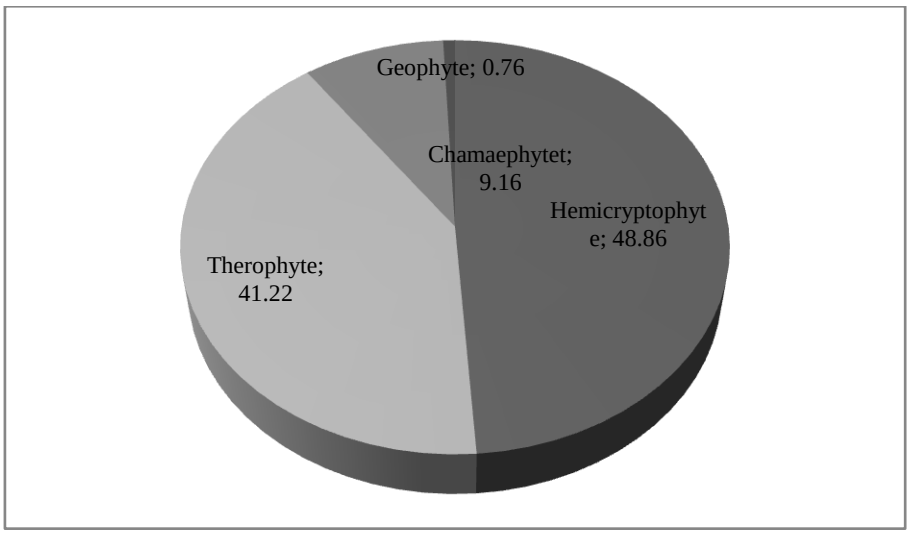

FIG. 5: Distribution of life form in abondened pasture units (%)

As can be seen in figure 4, hemicryptophytes were dominant in the investigated area, followed by therophytes, chamaephytes and geophytes. Species having hemicryptophyte life forms in the vegetation biological spectrums were in 48.86 %, and plants with therophyte life forms were 41.22 %, chamaephytes 9.16 %, and geophytes 0.76 % (Figure 5). Tuna (2010) pointed out that determining quantitative plant diversity of natural pastures is very important. Therophyte recruitment

depends on seed banks for population survival, and as they have a short life cycle they are more responsive to environmental and biotic changes than other life forms (Vila‘1 et al.2006). Because of low rainfall and continuous drought, they finished their life cycle in a short time (Mood 2008) Annual plants which had the rophyte life form were less abundant in vegetations as the height grew up above the sea level. The research area is also situated sea level. Similar results were stated by Cerit ve Altın 1999; Tuna 2000; Tuna 2010) . According to Raunkiaer (Akman and Ketenoglu, 1992) the biological spectrum is an indicator of climate and the total flora of Mediterranean countries contain30% hemicryptophytes and 40% therophytes, whereas other countries (far from the Mediterranean) contain 50% hemicryptophytes, 30% cryptophytes an 20% therophytes.

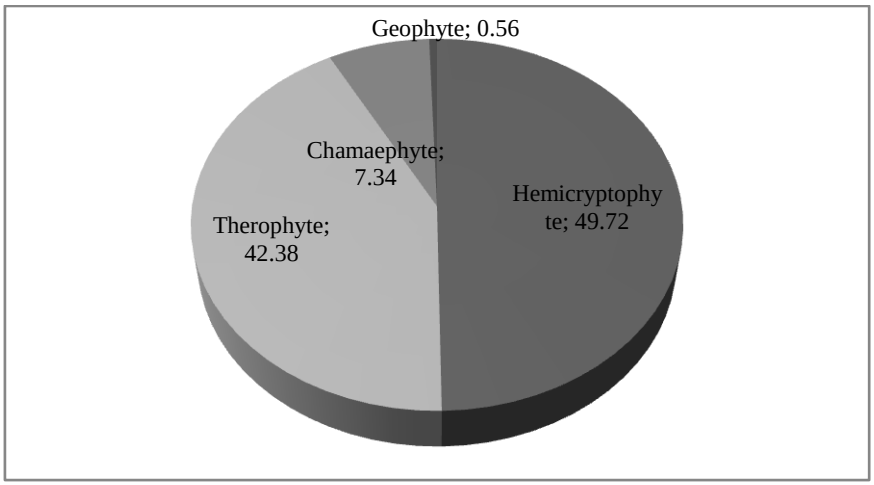

FIG. 6: Distribution of life form in grazed pasture units (%)

As can be seen in figure 6, hemicryptophytes were dominant in the grazed unit, followed by therophytes, chamaephytes and geophytes. Species having hemicryptophyte life forms in the vegetation biological spectrums were in 49.72%, and plants with therophyte life forms were 42.38 %, chamaephytes 7.34 %, and geophytes 0.56 % (Figure 6). According to Box (1981), the study of plant life forms is important, because it provides the basic structural components of vegetation stands and explaining vegetation structure. Hemicryptophytes includes the species of perennial plants. Meanwhile, these species are the feed plants producing quality feeds and are among the plants which animals prefer. Grazing caused changes in species composition in the native pasture (Milchunas and Lauenroth 1993, Milton et al. 1994, Pykälä 2004, Mosallam 2007), especially palatable plants eaten by livestock. Hence, heavy grazing pastures may not demonstrate original plant diversity. In addition to, the relation between human activities and disturbance (some anthropogenic and grazing) effects and the increase of therophytes were reported (Grime, 2001; Naqinezhad et al., 2006; Ravanbakhsh et al. 2007).

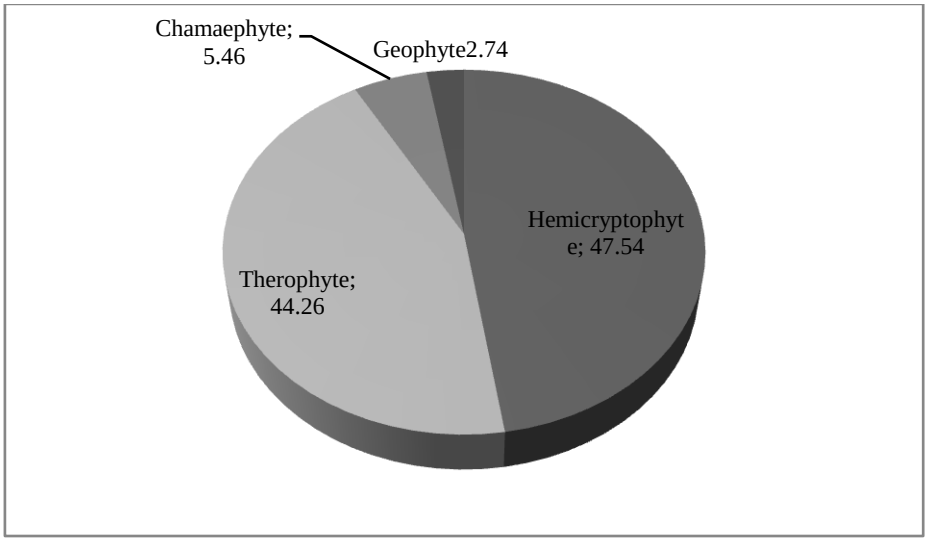

FIG. 7: Distribution of life form in mowed pasture units (%)

As can be seen in figure 6, hemicryptophytes were dominant in the mowed area, followed bytherophytes chamaephytes and geophytes. Species having hemicryptophyte life forms in the vegetation biological spectrums were in 47.54%, and plants

with therophyte life forms were 44.26%, chamaephytes 5.46 %, and geophytes 2.74 % (Figure7). Braun- Blanquet (1964), reported that grass and pasture plants have usually hemicryptophytes life form. In other a study, Panahy et al. *(*2008) stated that, the frequency of hemicryptophytes and therophytes among the plants of the region showed that the effect from two types of climate-Mediterranean and cold temperate-affected them. Additionaly, therophyte proportion were calculated higher than other units. Therophytes which *Aegilops triuncialis*, *Avena fatua*, *Vulpia ciliata* were found dominant ingrazed pasture units while therophytes like as *Trifolium campestre*, *Medicago minima* and *Lotus corniculatus* were determined common in mowed pasture units.

3.4 Life Span of Pasture Species

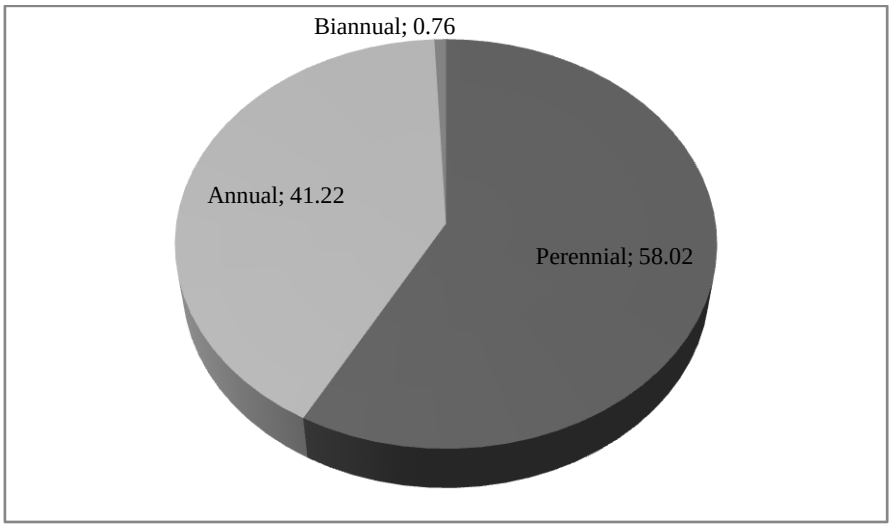

FIG.8: Distribution of life span in abondened pasture units (%)

In the assessment of life span, the dominant life span are perennials, which constitute 58.02 % of studied flora, followed by annuals (41.22%), and biannuals (0.76%) in the protected sites. (Figure 8) . It was concluded that annual species are resistant to grazing are more common in the sites that are exposed to heavy grazing. Annual or perennial herbaceous plants that easily spread, easily settle and quickly develop grow especially in areas that are plowed and abandoned (Holechek et al. 1989). In addition to, McIntyre and Lavorel (1994) found significant associations between individual plant species and various environmental and grazing-related factors in Australian range and Vegetation in the grazed treatments was dominated by short annual grasses and annual thistles, while under long-term protection from grazing tall annual and perennial grasses were dominant (Golodets et al. 2010) protected areas were dominated by tall perennials and tall annual grasses.

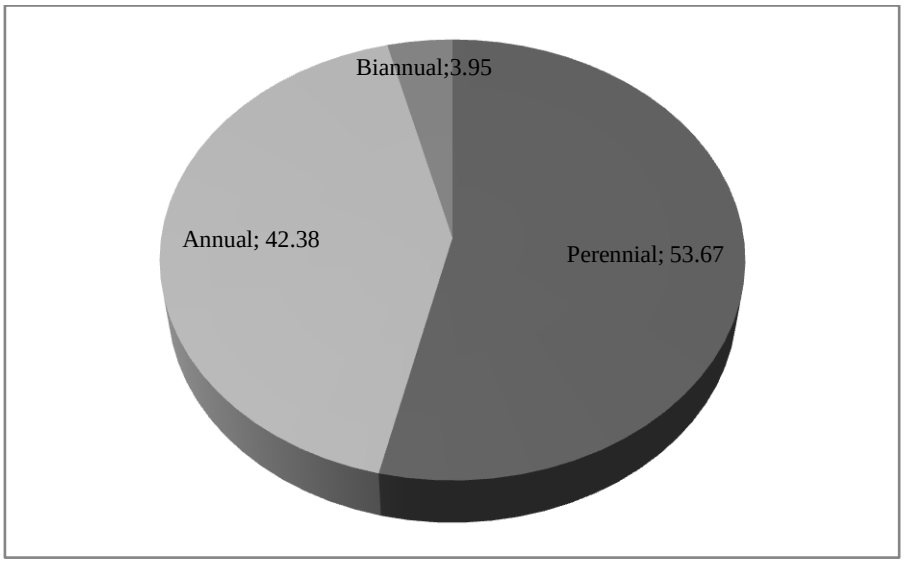

FIG.9: Distribution of Life span in grazed pasture units (%)

In the assessment of life span, the dominant life span are perennials, which constitute 53.67 % of studied flora, followed by annuals (42.38%), and biannuals (3.95%) in the grazing sites (Figure 9). Annual species may tolerate grazing due do their

fast growth and early and often prolific seed set. Fabaceae in grasslands are often therophytes with a mesomorph leaf anatomy and persistent seed bank (Dupre and Diekmann 2001). Arid and semi-arid regions are often characterized by an abundant flora of annual plants that complete their life cycle within a relatively short favourable growth period (Alhamad 2006). Similar to earlier reports by Smith (1994) and Snyman (1998), grass species are important as an indicator pasture, as species may vary significantly in their acceptability to grazing herbivores, not only due to phonological differences.

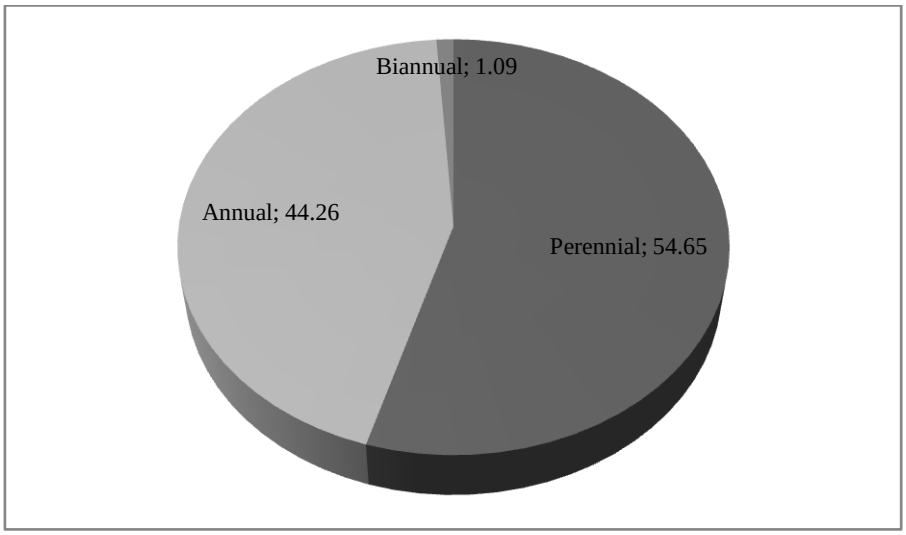

FIG. 10: Distribution of life span in mowed pasture units (%)

In the assessment of life span, the dominant life span are perennials, which constitute 54.65 % of studied flora, followed by annuals (44.26%), and biannuals (1.09%) in the Mowed sites(Figure 10). *Lolium perenne, Trifolium repens, Chrysopogon gryllus , Vicia villosa, Carex flacca, Trifolium* were determined as dominant in mowed pasture units. (Table 1). These plants are perennial. Meanwhile, *Lolium perenne, Trifolium repens , Carex flacca* more common in humid area than dry area .A similar result for Carex flacca was determined by Tölgyesi et al. (2015).According **to** Altın (1992), *Lolium perenne* is a graminae that prefers damp, fertile and heavy soils .

3.5 Pytogeographical Elements of Pasture Species

Together with this, pytogeographical analysis of the study area showed the presence of two uni-regional chorotypes (pytogeographical groups), namely Mediterranean, and Euro-Siberian in addition to multiregionalor is not known groups.

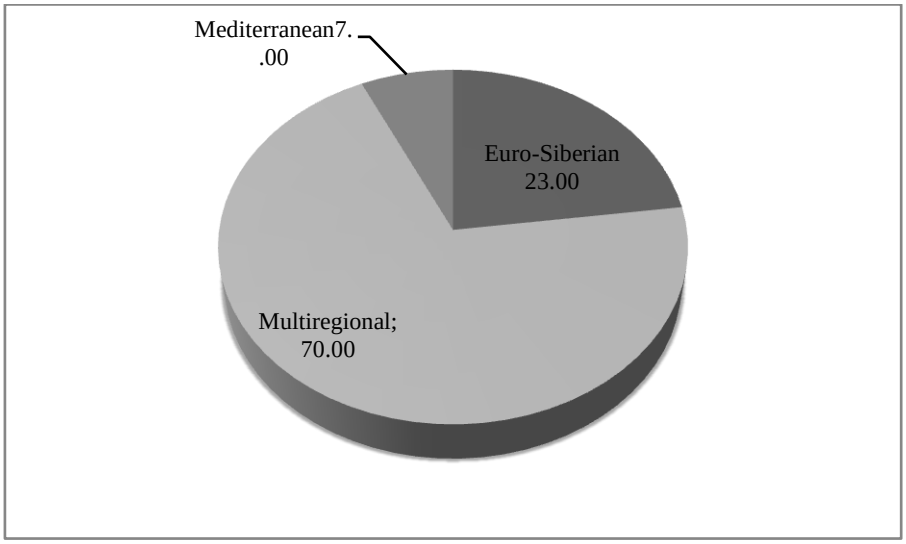

FIG. 11: Distribution of pytogeographical region in abondened pasture units (%)

The distribution of species according to phytogeographical regions is given in Figure 2. Distribution of the taxa according to phytogeographical regions, is follows; 23.00 % Euro-Siberian, 7.00 % Mediterranean, and the ratio of the type which is unknown region and or multi regionals is 70.00% (Figure 11)As the researchers like Zohary (1973) and Donmez (1968) said,

especially parts of coasts consist of elements having Mediterranean vegetation. Cerit and Altin (1999) also said that the research area in Tekirdag is under the effect of Europe-Siberian and Mediterranean elements. Donmez (1968) said that Mediterranean vegetation is seen in the southwest of Trakya (Thrace), in the district of Tekirdag and in the northeast part of Black Sea coast. Deniz and Sumbul, (2004), point out that Turkey serves as a bridge between southwest. Asia and south-east Europe, and little information about the distribution of some species is available, the percentages of multiregional species and those of known phytogeographical origin in Turkey are generally high.

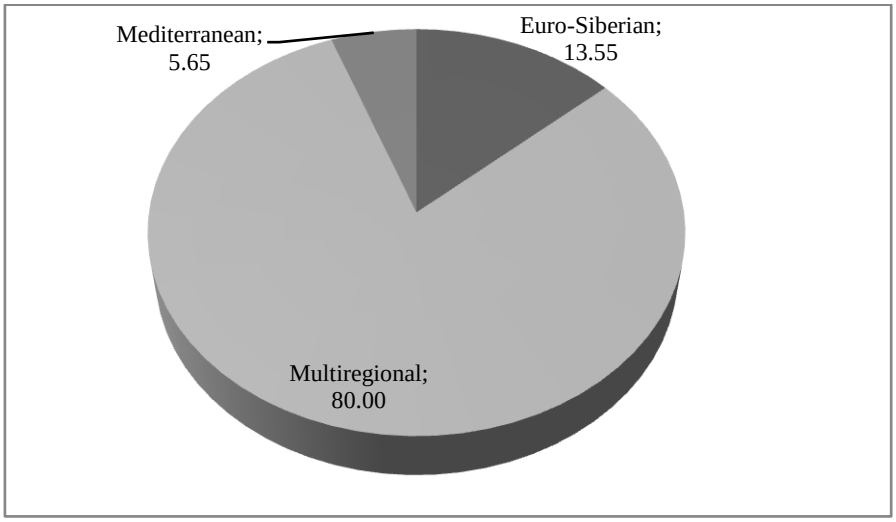

FIG. 12: Distribution of pytogeographical region in grazed pasture units

The distribution of species according to phytogeographical regions is given in Figure 12. Distribution of the taxa according to phytogeographical regions, is follows; 13,55 % Euro-Siberian, 5.65% Mediterranean, and the ratio of the type which is unknown region and or multi regionals is 80.8% (Figure 12).According to; Heithschmidt and Stuth, (1991) the climate, intense grazing pressure can also be a determinant factor for the relative abundance and geographic distribution of different life forms.

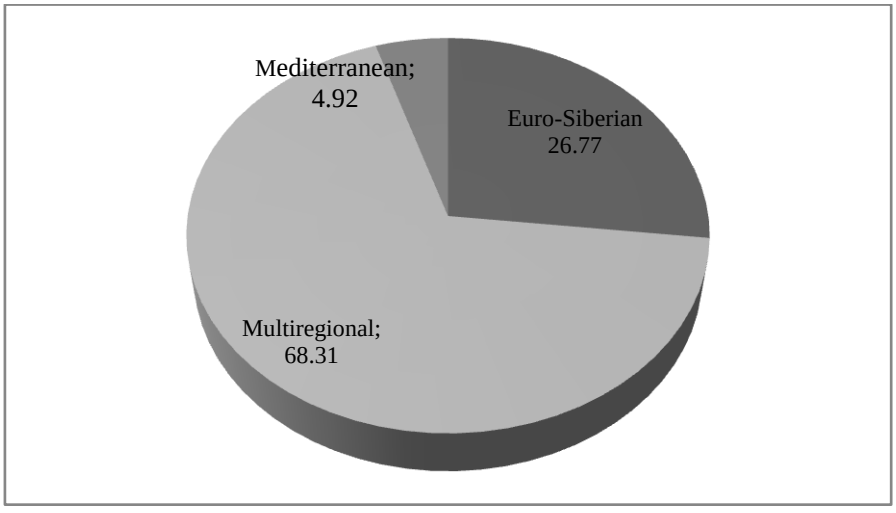

FIG. 13: Distribution of pytogeographical region mowed pasture units (%)

Distribution of the taxa according to phytogeographical regions, is follows; 26,77 % Euro-Siberian, 4,92 % Mediterranean, and the ratio of the type which is unknown region and or multiregionals is 68,31% in mowed pasture (Figure 13).The level of Euro –Siberian elements were determined highest other pasture sites in research area. Europe-Siberian elements prefer usually humid regions and watery and marshy habitats. The majority of elements spreading large fields can arise from the fact that some species of which tolerance are extreme have spread out largely in similar climate and soil conditions (Tuna 2010).In my previous study, distribution of the taxa according to phytogeographical regions, is follows; 13.2% Euro-Siberian, 24.5% Mediterranean, and the ratio of the type which is unknown region and or multiregionals is 62.3 % in Trakya region (Tuna, 2000).

IV. CONCLUSION

The results indicate that species composition changes by grazed, abondened, mowed .It was determined that the diversity can get better in pastures with corrupt vegetation by protecting them against heavy grazing.Pasture management strategies change based on species compositions and, therefore, this information should be available to select for future pasture management decisions . The combination of the frequency, families, life form and life span, phytogeographical region of species identifies of plants that have different importance depending on the duration of land practices, like the increase or reduction in grazing pressure (Tuna 2010). In other words, this quantitative study has the potential to ensure sample for developing suitable management techniques for semiarid and arid region pastures.

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