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Review Article

Carbon dioxide metabolism and ecological significance of enzyme complex systems in terrestrial ecosystem

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ABSTRACT

The growing concern for global climate change due to increased atmospheric greenhouse gas (GHG) raises the challenge of finding novel technological approaches to stabilize GHG. Rise in CO_2 emissions due to anthropogenic activities impinge interconnected ecosystem issues. Biological CO_2 mitigation through biological fixation is considered a promising and eco-sustainable method that can be exploited further. In order to reduce atmospheric CO_2 there is need of urgent CO_2 mitigation strategies. Microbial groups including cyanobacteria, green algae and many autotrophs could potentially fix CO_2 more efficiently than higher plants, due to their higher metabolic rate. Some examples of the potential of biological-CO_2 mitigation are reported and discussed in this paper. This review addresses different enzyme complex system prevalent in the ecosystem like calvin cycle, reductive carboxylic acid cycle, reductive acetyl-coenzyme A pathway, and hydroxypropionate cycle. Review critically addresses ecological significance of different CO_2 trapping enzyme complex systems like Rubisco, phosphoenolpyruvate carboxylase, carbonic anhydrase, and methanogenic enzymes. Paper concludes highlighting potential use of these complex enzyme systems for possible approaches mitigating global climate change.

Keywords: Climate change, CO_2, Enzymes, CO_2 sequestration, Terrestrial ecosystem.

1. INTRODUCTION

Carbon cycle is the backbone of ecosystem processes and drives the flow of essential elements from the environment to living system and back to the environment again. CO_2 fixation is the primary process of utmost importance for existence of life as it involves conversion of inorganic carbon to organic forms which are the base of biological structures in our living system. This conversion is generally catalysed by energy either in the form of light or chemical energy. Plants, cyanobacteria and algae are the major contributor for CO_2 assimilation. Microbial CO_2 fixation also contributes a significant proportion of total biological carbon transformation. In a well balanced ecosystem, carbon capture through photosynthesis, carbon deposition in the soil and ocean sediments, and carbon emission from biological and geological sources are in equilibrium. Since the beginning of the industrial age, however, this balance has been disturbed due to fossil fuel use

and global deforestation. Due to industrialization atmospheric CO_2 is rapidly increasing and the excess contributes significantly to global warming [1, 2]. Thus, there is need of better efficient approaches to sequester atmospheric CO_2. Biological CO_2 capture systems are one of innovative approaches to improve the CO_2 capture efficiency. In this review we have attempted to discuss different CO_2 fixing enzymatic process and their ecological significance.

2. AUTOTROPHIC CARBON FIXATION PATHWAYS

2.1. Calvin cycle

Calvin cycle, also known as Calvin-Benson-Bassham (CBB) cycle, where CO_2 reacts with the five carbon sugar ribulose 1,5-bisphosphate (RuBP) to yield two carboxylic acids, 3-phosphoglycerate, which is converted to sugar [3]. This cycle is active in plants, algae, cyanobacteria, some aerobic or facultative anaerobic Proteobacteria, CO-oxidizing mycobacteria and representatives of the genera *Sulfobacillus* (iron- and sulphur-oxidizing Firmicutes) and *Oscillochloris* (green sulphur bacteria) (Table 1). The presence of the key enzyme, ribulose 1,5-bisphosphate carboxylase-oxygenase (RubisCO), is generally considered as indication of autotrophic metabolism.

2.2. Reductive citric acid cycle

This autotrophic cycle is also known as Arnon-Buchanan cycle [4], reported in the green sulphur bacterium *Chlorobium limicola*, and also called as reductive citric acid cycle [5]. This cycle is less energy-consuming than the Calvin cycle, involves enzymes that are sensitive to oxygen. Therefore, found only in anaerobes or in aerobes growing at low oxygen tensions. These include some Proteobacteria, green sulphur bacteria and microaerophilic bacteria of the early bacterial phylum Aquificae and in certain archaea (notably *Thermoproteus neutrophilus*) [6].

Table 1. Characterization of CO_2 fixing metabolic pathways, enzymes and ecological distribution.

Metabolic pathways	Respiration	Ecology	Enzyme system
Calvin cycle	Aerobic and facultative anaerobic	Plants, algae, cyanobacteria, Proteobacteria, Mycobacteria, *Sulfobacillus*, Firmicutes and *Oscillochloris*	RubisCO
Citric acid cycle	Low oxygen tension or anaerobic	Proteobacteria, green sulphur bacteria, *Thermoproteus neutrophilus*	2-oxoglutarate synthase, isocitrate ATP-citrate, lyase dehydrogenase, pyruvate synthase, PEP carboxylase
Acetyl co A pathway	Anaerobic	Planctomycetes, Spirochaetes and Euryarchaeota	acetyl-CoA synthase-CO dehydrogenase, formylmethanofuran dehydrogenase, pyruvate synthase
3-Hydroxypropionate cycle	Anaerobic	Green non-sulphur bacteria, Chloroflexaceae	acetyl-CoA and propionyl-CoA carboxylase
Hydroxypropionate hydroxybutyrate cycle	Aerobic	Crenarchaeota, Sulfolobales	acetyl-CoA and propionyl-CoA carboxylase
Dicarboxylate hydroxybutyrate cycle	Anaerobic	Crenarchaeal orders, Thermoproteales and Desulfurococcales	pyruvate synthase, PEP carboxylase

2.3. Reductive acetyl-coenzyme A pathway

Third type of autotrophic pathway was discovered in certain Gram-positive bacteria and methane-forming archaea. Also known as reductive acetyl-coenzyme A (acetyl-CoA) or Wood-Ljungdahl pathway [7]. Anaerobic organisms including Proteobacteria, Planctomycetes, spirochaetes and Euryarchaeota exhibit this C metabolic pathway. Here, one CO_2 molecule is reduced to CO and other to a methyl group. Subsequently, acetyl-CoA is synthesized from CO and the methyl group. This pathway is the most energetically favourable autotrophic carbon fixation pathway. However, it is restricted to strictly anaerobic organisms. Many organisms representing autotrophic Euryarchaeota are strictly confined to anoxic conditions. These organisms are specialized in metabolizing C1 compounds and/or acetate, with low ATP yields. These limitations favour reductive acetyl-coenzyme A pathway [8].

2.4. 3-Hydroxypropionate bicycle

The 3-hydroxypropionate bicycle is aunique pathway generally found in some green non-sulphur bacteria of the family Chloroflexaceae [9, 10]. The conversion of acetyl-CoA plus two bicarbonates to succinyl-CoA uses the same intermediates as in the hydroxypropionate-hydroxybutyrate cycle, but enzymatic system is different. Furthermore, the regeneration of acetyl-CoA proceeds by the cleavage of malyl-CoA, yielding acetyl-CoA and glyoxylate.

2.5. Hydroxypropionate-hydroxybutyrate cycle

The hydroxypropionate-hydroxybutyrate cycle occurs in aerobic Crenarchaeota (Sulfolobales and possibly marine Crenarchaeota group I) [6, 11, 12]. Although some of the intermediates and the carboxylation reactions are the same as in the 3-hydroxypropionate bicycle in Chloroflexaceae, the archaeal cycle probably has evolved independently.

2.6. Dicarboxylate-hydroxybutyrate cycle

The dicarboxylate-hydroxybutyrate cycle occurs in the anaerobic crenarchaeal orders Thermoproteales and Desulfurococcales. The enzyme 4-hydroxybutyryl-CoA dehydratase, 4-hydroxybutyryl-CoA is dehydrated to crotonyl-CoA. Beta-oxidation of crotonyl-CoA leads to two molecules of acetyl-CoA. Thus, the cyclic pathway forms an extra molecule of acetyl-CoA, with pyruvate synthase and PEP carboxylase as the carboxylating enzymes [9, 11, 13].

3. FUNCTIONALLY SIGNIFICANT ENZYMES

3.1. Ribulose-1,5-bis-phosphate carboxylase

Rubisco is an important enzyme in the biosphere by which autotrophic bacteria, algae, and terrestrial plants survives by fixing CO_2 into organic biomass [14]. Rubisco catalyses the primary photosynthetic CO_2 reduction reaction, the capturing of atmospheric CO_2 to ribulose-1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate (3PGA), which is subsequently used to build the organic molecules of life. Rubisco also constitutes up to half of the soluble protein in the plant leaf [15]. Global carbon cycle by it in oceanic phytoplankton is estimated to provide more than 45% of global net primary production per year. Carbon fixation resulting from Rubisco's activity forms more than 1011 tons of atmospheric CO_2 annually [16]. Its catalytic activity is chiefly associated with the central part of the Calvin-Benson--Basham (CBB) reductive pentose phosphate pathway. Rubisco is considered to be the most abundant enzyme on our planet and is therefore recognized as the major entrance through which inorganic carbon (CO_2) enters into our living system. Besides the photosynthetic eukaryotic organisms, numerous prokaryotes have also been found to rely on the Calvin cycle for CO_2 fixation, and many more have been shown to at least harbour a Rubisco. Rubisco requires prior activation by carbamylation of the e-amino group of active-site Lys201 by a CO_2 molecule to become functional, which is distinct from the substrate CO_2 [17]. The carbamylated Lys201 further requires stabilization by the binding of Mg ion to the carbamate. It seems that the Calvin cycle is utilized by purple nonsulfur bacteria (*Rhodobacter*, *Rhodospirillum*, *Rhodopseudomonas*) and purple sulfur bacteria (*Chromatium*), cyanobacteria (*Synechococcus*, *Anacystis*, *Anabae-*

na), along with hydrogen bacteria (*Ralstonia*, *Hydrogenovibrio*) and other chemoautotrophs (*Thiobacillus*). Rubisco enzyme found in archaea, bacteria, and eukaryotes are described in Table 1. Different types of Rubisco are highlighted in Table 2. Of these, only form I, II, and III have been shown to have RuBP dependent CO_2 fixing ability [18, 19]. Form IV enzymes have been termed 'Rubisco-like' proteins and have been shown to be functional in the methionine salvage pathway in many bacteria [18]. There are suggestions that Form IV Rubisco may have been the evolutionary progenitor of CO_2 fixing Rubisco as it is known today [19]. Form III Rubiscos have to date only been found in archaea, and their CO_2 fixing role has been somewhat uncertain due to the lack of an identifiable phosphoribulokinase in archaea [20], although some archaea have recently been identified with potential phosphoribulokinase genes [21]. These enzymes are found in a wide range of chemo-, organo-, and phototrophs occurring in bacteria, algae, and plants. Form I enzymes show considerable diversification and have been divided into four distinct clades. Forms IA and B belong to a 'Green' grouping and are found in proteobacteria, cyanobacteria, green algae, and higher plants. Forms IC and D are members of a 'Red' group, occurring in proteobacteria and non-green algae. Form IA enzymes are divided into two distinct types, IAc and IAq, based on two distinct types of small subunits and gene arrangements. Form IB enzymes have been subclassified into IB and IBc. Form IBc is found in cyanobacteria [22].

Table 2. Rubisco complex types, metabolic pathways and ecological distribution.

	Type I **Green IA of IAc, 1Aq; IB of IB or IBc; Red IC or ID**	**Type II**	**Type III**	**Type IV**
Pathway involved	CBB	CBB	RuPP	Methionine salvage
Ecology	Cyanobacteria (IAc), proteobacteria (IAc and IAq) Cyanobacteria (IBc), higher plants (IB), Heterokont and haptophyte algae (ID)	Proteobacteria, archaea, and dinoflagellate algae	Archaea	Bacteria, archaea, including both photosynthetic and non-photosynthetic bacteria

CBB - Calvin-Benson-Bassham pathway.

The enzyme consists of two distinct subunits, a large (L) subunit (50-55 kDa), which is the catalytic subunit of the enzyme, and a small (S) subunit (12-18 kDa). Dimerization of two L subunits is necessary to generate two catalytic centers per L2 dimer, and the quaternary structure of a Type I enzyme is L8S8. In eukaryotic cells, the L subunit is encoded on the chloroplast genome, while the genes for the S subunit are located on the nuclear genome. In contrast to Type I, the Type II enzyme has been identified in only a relatively small number of bacteria. This enzyme is composed solely of L subunits and is usually found in an L2 form. *Rhodospirillum rubrum* and several *Rhodobacter* sp. harbors single Type II Rubisco [23]. *Thiobacillus* [24] and *Hydrogenovibrio* [25] have been found to contain both Type I and Type II Rubisco.

Rubiscos are also known to possess oxyenase activity, in which a O_2 molecule, compete with CO_2 for the enzyme's active site. This reaction forms 3-phosphoglyceric acid and phosphoglycolate. The latter product is subsequently oxidatively metabolized via photorespiration, leading to a net loss in CO_2 fixation. The ability of a particular Rubisco to discriminate between carboxylation and oxygenation is an intrinsic property, often evaluated by determining its specificity factor (τ value). This is defined as $VCO_2KO_2/V\ O_2KCO_2$, where VCO_2 and VO_2 refers to the maximal velocity for CO_2 and O_2, respectively, and KCO_2 and KO_2 refers to respective Michaelis constants. Atmospheric CO_2 levels have decreased, and O_2 has increased over evolutionary time, thus τ has increased in more recently derived organisms in response to the

selective pressures. Type I Rubiscos from higher plants such as spinach display a ι value of approximately 80, while those from bacteria or green algae exhibit values between 40 and 60. The τ values of Type II Rubiscos are even lower, ranging from approximately 10 to 20 [26].

The Calvin cycle provides two distinct functions in *R. capsulatus* depending on the growth conditions. The cycle in autotrophically grown cells contributes to the fixing of CO_2, in the usual manner. However, under photoheterotrophic conditions, *R. capsulatus* utilizes the Calvin cycle in order to release excess reducing equivalents to the preferred electron acceptor, CO_2 [27]. *R. capsulatus* harbors two distinct types of Rubisco, the Type I enzyme encoded by the cbbL and cbbS genes in the cbb I operon, and the Type II enzyme encoded by the cbbM gene in the cbb II operon [23]. The Type I Rubisco is the predominant during photoautotrophic growth, and is not synthesized during photoheterotrophic growth. The Type II Rubisco is produced under all growth conditions and can be supposed to contribute in balancing the redox equilibrium of the cell during photoheterotrophic growth.

Recent studies have indicated that although a functional Calvin cycle has not yet been identified in archaea, some hyperthermophilic strains harbor proteins that exhibit Rubisco activity. These species are *Thermococcus kodakaraensis* KOD1 [28-32] *Methanococcus jannaschii* [33], and *Archaeoglobus fulgidus* [33]. *T. kodakaraensis* grows at temperatures between 65°C and 100°C, obligatory anaerobic, and exhibits heterotrophic growth on amino acids, starch, oligosaccharides, and pyruvate. An open reading frame (ORF) of *T. kodakaraensis* KOD1 display ~50% similarity to Type I and Type II Rubiscos [28]. Similar ORFs have also been found on the genomes of *M. jannaschii* and *A. fulgidus*. This suggested existence of some kind of physiological role for these genes in hyperthermophilic archaea. Phylogenetic analysis of Rubisco sequences indicates that the archaeal Rubiscos, cluster together in a branch distinct from previously identified Type I and Type II enzymes.

3.2. Phosphoenolpyruvate carboxylase

Cyanobacteria, algae and C3 plants also contain another enzyme which fix C, phosphoenolpyruvate carboxylase (PEPc). PEPc is the main enzyme responsible for the C fixation during photosynthesis in C4 and CAM plants [34, 35]. In cyanobacteria, this enzyme is responsible for fixating 20% of the total carbon. It is essential as it plays an important anaplerotic role [36]. PEPc fix C to produce oxaloacetate which is an intermediate in the TCA cycle. Thus, cyanobacteria mainly fixate C into the C3 cycle but they also contain the C4 pathway [36, 37]. The main problem in aquatic systems, where most of cyanobacteria live, is the inorganic C (Ci) availability. The equilibrium between CO_2 and HCO_3^- is slow in pH between 7 and 8.5. However, cyanobacteria have developed different transporters in order to uptake inorganic C (Ci) efficiently when high or low levels are available [38, 40]. In addition, it seems that light is a prerequisite for the expression of CO_2 response genes [40]. Three different phosphoenolpyruvate enzymes including phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase and phosphoenolpyruvate transphosphorylase have been found in nature. These enzymes perform the β-carboxylation of PEP in order to lead to oxaloacetate. The main difference among them is the inorganic phosphate acceptor [41]. This enzyme is present in bacteria, alga and vascular plants [35, 41]. PEP carboxylases have been purified from a wide range of bacterial sources. All these carboxylases are tetramers with subunit masses of between 90 and 110 kDa [42-44]. PEP carboxylases are sensitive to fatty acids, acetyl-CoA and fructose-1,6-bisphosphate as activators and aspartate and malate as allosteric inhibitors. In plants, PEP carboxylases additionally are subject to regulation by reversible phosporylation modification a process so far not detected in bacteria. A new type of PEP carboxylase called PEP carboxylase A with no discernible evolutionary relationship to the hitherto known enzymes has been described for the archaeon *Methanothermobacter thermoautotrophicus* [45]. No PEPC homologs have been detected in *Archaea* thus far. However, PEPC activities have been measured [46] and the corresponding enzymes were purified and biochemically characterized in two archaeal hyperthermophiles: *Methanothermus sociabilis* [47] and *Sulfolobus acidocaldarius* [48]. It has been shown that the archaeal enzyme resembles the bacterial PEPC in quaternary structure

which requires Mg ion for its activity. However, unlike the bacterial enzyme, the archaeal PEPC is considerably smaller in size and lacks some typical regulatory properties.

3.3. Carbonic anhydrase

In environment, CO_2 is in equilibrium with HCO_3, carbonic acid and carbonate of which HCO_3^- is the most physiologically important. HCO_3^- is negatively charged and highly soluble in aqueous solution but poorly soluble in lipids, while CO_2 is highly soluble in both aqueous solution and lipids. Therefore, CO_2 can freely diffuse in and out of the cell, HCO_3^- can be transported across the cell membrane. Conversion of HCO_3^- to CO_2 may facilitate its transport into the cell while conversion of CO_2 to HCO_3^- is important for trapping CO_2 in the cell. Thus, enzymatic conversion of CO_2 and HCO_3^- not only allows the cell to concentrate CO_2 to the levels required for cellular enzymes but also helps the cell maintain the proper intracellular levels of CO_2 and HCO_3^- to carry out cellular processes.

Carbonic anhydrase (CA) is a Zn-containing enzyme that catalyzes the inter-conversion of CO_2 and HCO_3^- [49, 50]. CA is fundamental to various biological functions including photosynthesis, respiration, and CO_2 transport. Cyanobacteria and some chemoautotrophic bacteria are able to grow in environments with limiting CO_2 concentrations by employing a CO_2-concentrating mechanism. The efficiency of CO_2 fixation by the sequestered RubisCO is enhanced by co-localization with a specialized carbonic anhydrase that catalyzes dehydration of the cytoplasmic bicarbonate and ensures saturation of Rubisco with its substrate.

There are five distinct CA families (α, β, γ, δ and ζ). These families have no significant similarity in amino acid sequence i.e. evolutionarily unrelated. Alpha types are present in vertebrates, bacteria, algae and cytoplasm of green plants [49, 51-54]. Beta type are present predominantly in bacteria, algae and chloroplasts of both mono- as well as dicotyledons. β-CA have been obtained from a red alga (*P. purpureum*), aplant chloroplast (*P. sativum*), bacterium (*E. coli*), an Archaea (*M. thermoautotrophicum*), two enzymes from pathogenic bacteria (*M. tuberculosis*), acarboxysome (*H. neapolitanus*), gram negative bacteria (*Haemophilus influenzae*) and so on. Gamma type CA are mainly found in Archaea and some in bacteria. These types have been isolated from the methanogenic archaeon Methanosarcina thermophile growing in hot springs. The carbonic anhydrase (Cam) from *M. thermophila* is the prototype of a novel gamma class [55]. Sequences with identity to Cam have been previously identified in all three domains of life, but the proteins encoded by these sequences have yet to be examined for carbonic anhydrase activity. Delta class has been found in diatoms. Zeta type is found in the species *Thalassiosira weissflogii*. The high efficiency of CA is fundamental to many biological processes like photosynthesis, respiration, pH homeostasis, ion transport, water and electrolyte balance, etc. CA has the ability to catalyze the hydration of 600,000 molecules of CO_2 per molecule of CA per second comparable to a theoretical maximum rate of 1,400,000 [56, 57]. Recently, CA has been considered as an important biocatalyst for CO_2 sequestration technology because the accumulation of CO_2 is the main cause for global climate change and it is critical to develop technologies that can reduce atmospheric CO_2 level [58-60].

3.4. Methanogenic pathway utilizing CO_2

About 1% of the plant materials formed by photosynthesis from CO_2 are remineralized via CH_4. More than $l0^9$ t of the combustible gas is intermediately generated per year [61]. The organisms, which produce CH_4, belong to the archaea. In the domain archaea, methanogens are relatively widely spread in the kingdom Euryarchaeota.

Methanogens are classified in five orders: *Methanopyrales, Methanococcales, Methanobacteriales, Methanosarcinales* and *Methanomicrobiales* [62]. Most of the methanogens can utilize CO_2 and H_2 as substrates for methanogenesis. CO_2 is bound to the first C, carrier methanofuran and reduced to formyl-methanofuran. The formylation reaction is catalyzed by formylmethanofuran dehydrogenase (Fmd). Fmd contains molybdopterin and iron-sulfur [63-65]. There are two isoenzymes, the first contains molybdenum as a molybdopterin while the other is the tungsten analogue.

3.5. Tetrahydromethanopterin formyltransferase (Ftr)

The formyl group on formylmethanofuran is transferred to the second C1 carrier, tetrahydromethanopterin (H4MPT). This reaction is catalyzed by formylmethanofuran H4MPT formyltransferase (Ftr) [66-68]. The enzyme has been found in *Methanothermobacter thermoautotrophicus*, *Methanosarcina barkeri*, *Methanopyrus kandleri*, *Methanothermus fervidus* and the sulfate reducing archaeon *Archaeoglobus jiilgidus* [61, 66-71].

3.6. Methylene-H_4MPT dehydrogenase (Mtd)

N^5,N^{10}-methenyl-H4MPT is reduced by the methylene-H_4MPT dehydrogenase reaction, yielding N^5,N^{10}-methylene- H4MPT. There are two types of methylene-H_4MPT dehydrogenase: an F_{420}-dependent enzyme and an F_{420}-independent enzyme [72]. F_{420} is a coenzyme for hydride transfer, which has been originally found in methanogens [73]. F_{420}-dependent methylene-H_4MPT dehydrogenase (Mtd) catalyzes the reversible reduction of N^5,N^{10}-methenyl-H_4MPT and reduced F_{420}($F_{420}H_2$) to N^5,N^{10}-methylene-H_4MPT. The reduced $F_{420}H_2$ is regenerated by the reaction catalyzed by F_{420}-reducing hydrogenase [65]. The F_{420}-independent methylene-H_4MPT dehydrogenase, which is designated as H_2 forming methylene-H_4MPT dehydrogenase (Hmd), catalyzes the reversible reduction of N^5,N^{10}-methenyl-H_4MPT and molecular hydrogen to N^5,N^{10} methylene- H_4MPT [74, 75]. Followed by, N^5,N^{10}-methylene-H_4MPT is reduced to N^5-methyl-H_4MPT at expense of $F_{420}H_2$ by methylene- H_4MPT reductase [61]. Subsequently, the methyl group of N5 methyl-H4MPT is transferred to coenzyme M by the reaction catalyzed by methyl-H4MPT:coenzyme M methyltransferase (Mtr) [76] (Table 3).

Table 3. Carbonic anhydrase type, classification and ecological significance.

Families	Type and Location	Ecology
Alpha	Cytosol - I, II, III, VII, XIII; Mitochondria - VA, VB; Secretions - VI; Membrane - IV, IX, XII, XIV, XV	Vertebrates, Bacteria, Algae and cytoplasm of green plants
Beta	Cytosol - EC (*Escherichia coli* CA), MT (*Methanobacterium thermoautotrophicum*); Chloroplast - PS (*Pisum sativum*)	Red alga (*P. purpureum*), Plant chloroplast (*P. sativum*), E. coli, Archaea (*M. thermoautotrophicum*), *Mycobacterium tuberculosis*, *Haemophilus neapolitanus*, *Haemophilus influenza*
Gamma	Cytoplasm	Archaea and Bacteria
Delta	Cytoplasm	Marine diatom - *Thalassiosira weissflogii*
Zeta	Cytoplasm	Chemolithotrophs, marine cyanobacteria containing carboxysomes and diatoms

4. CONCLUDING REMARKS

The best solution for climate change, as it relates to increased CO_2 levels, would be to increase nature's ability to absorb extra CO_2. The producers (photosynthetic organisms) of earth have the ability to fix carbon at a rapid rate. This review addressed different CO_2 fixing enzymes available in terrestrial ecosystem. CO_2 capturing molecule Rubisco has been well studied. But there are other CO_2 fixing enzymes abundant in the ecosystem and can be explored to regulate global climate change.

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AUTHORS' CONTRIBUTION

Manuscript was conceptualized and edited by SRM; GD drafted manuscript; KB compiled and formatted the manuscript to the need of journal's requirement; UA contributed in literature search during

the manuscript preparation.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest. Authors also declare no financial interest through this manuscript.

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Research Article

Studies on avifauna diversity of agronomy field of O.U.A.T Campus, Bhubaneswar, India

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ABSTRACT

Agronomy field of Orissa University of Agriculture and Technology, Bhubaneswar is an area of 32.6 hectare and a perimeter of 3.09 Km, forms an agro-ecosystem which is managed on regular basis by university authority for practical and field demonstration purpose for several agricultural researches and learning programs. Simple transect method and point count method was followed for observing the birds. Total 95 species of birds belonging to 43 families and 15 orders were recorded during a one year study period, from 15 March 2014 to 15 March 2015 at agronomy field of Odisha University of Agriculture and Technology, Bhubaneswar, Orissa, India. Being the first avifauna survey of the site, the present study indicates that the agronomy field of Orissa University of Agriculture and Technology, Bhubaneswar is sufficiently rich in avian species diversity and provides baseline information for future studies.

Keywords: Avifauna, Agro-ecosystem, Agronomy field, Diversity, Protection status.

1. INTRODUCTION

Indian subcontinent is very rich in biodiversity as a part of the Oriental biogeography regions. Out of the more than 9,000 birds of the world, the Indian subcontinent contains about 1,300 species, or over 13% of the world's birds [1, 2]. Birds are key component of an agro-ecosystem for maintaining ecological balance [3]. Birds constitute an important component of agro-ecosystems. The dual role of birds in agriculture is very well known [4, 5]. Avifauna is an important part of ecosystem which plays important role as a part of food web, indicator and also act as scavengers, pollinators, helps in seed dispersal and predators of insect-pest [6-8]. Agriculture provides a concentrated and highly predictable source of food to birds, which are (i) grain, seeds and fruits, (ii) green vegetation of the crop plants and grasses, and (iii) insects, other arthropods, rodents, etc., found in the soil, crops and other plants [9]. Since birds are integrated with farmer's activity as important and effective controller of insect-pest in agro-ecosystem, the extensive use of chemical insecticides and pesticides affects the bird population adversely through

harmful effects of chemicals and reduction in its regular prey [10].

The Agronomy Field of Orissa University of Agriculture and Technology (O.U.A.T), Bhubaneswar, Odisha, India (Fig. 1) is a well managed agro-ecosystem that provides a suitable habitat to many life forms as insects, amphibians, reptiles, fishes, mammals, birds, etc. There is a need to study the community structure and species diversity of birds of Agronomy field in order to investigate the impact of changing natural habitat and changing agricultural practices. Such studies will provide information on the species diversity of birds of the agronomy field of O.U.A.T, as there is dearth of information in literature on avian diversity of O.U.A.T campus, Bhubaneswar. In view of this, the present work was undertaken to study the species diversity of birds in the Agronomy Field of University.

2. MATERIALS AND METHODS

2.1. Study site

Agronomy field of O.U.A.T, Bhubaneswar (latitude 20°15'53.8"N and longitude 85°48'36.9"E) is located in the heart of Bhubaneswar city. It is an area of 32.6 hectare with a perimeter of 3.09 Km, managed on regular basis by university authority for practical and field demonstration purpose for several agricultural researches and learning programs.

2.2. Method

The site is studied for birds on regular basis from 15 March 2014 to 15 March 2015. Transect method, point count method and digital methods were followed to record the species, which is supplemented with field guide to identifying birds [1, 11-13]. Birds have been observed by using binocular of Olympus (10 x 50X), Tasco (8 x 25 mm) and photographs were taken by using digital camera model DSC-H9, DSC-H300 Sony and SX-400 IS Canon, respectively.

The survey was carried out twice a day from 6.00-8.00 am, during morning and 3.30-5.30 pm in afternoon regularly from 15 March 2014 to 15 March 2015. Three number of night survey was made for studying night birds in the month of February 2015.

Figure 1. Map of Agronomy Field of O.U.A.T, Bhubaneswar. Line(s) demarcated with Green and Orange color, represents the studied area.

Occurrence of the order/family of the bird is obtained by using the following formula:

$$\text{Percentage occurrence} = \frac{\text{No. of species of each order or family x 100}}{\text{Total no. of different family or species seen}}$$

Abundance (Number of sighting an individual species): Common (C), Un-common (U), Rare (R). Status (Migratory behavior of bird): Residential (RS), Local Migrant (LM), Migratory (M). Under Protection status, WPA: The Wildlife (Protection) Act 1972 of Government of India, Sch. IV: Comes and Protected Under Schedule IV of WPA: 1972, IUCN threatened category (Version-3.1, 2001): Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endanger (CR), Extinct in Wild (EW), Extinct (EX), CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora): Appendix-I (APP.I), Appendix-II (APP.II), Appendix-III (APP-III), NY-Not yet studied/comes under any of 3 appendices of CITES (I, II, III).

3. RESULTS

The results obtained from the present study showed 95 species of birds belonging to 15 orders and 43 families were present in agronomy field of O.U.A.T, Bhubaneswar. Out of all, order Passeriformes was found dominant (44.19% occurrence)

with 19 family of bird and order Podicipediformes, Gruiformes, Anseriformes, Columbiformes, Psittaciformes, Apodiformes, Cuculiformes, Falconiformes were found least occurred (2.35% occurrence each) with only 01 family of bird each (Figs. 2 and 3, Tables 1 and 2).

Under protection status Common Crow (*Corvus splendens*) comes under Schedule-V of The Wildlife (Protection) Act, 1972 and all 89 species of birds comes under Schedule-IV of the same Act [14]. However only one bird of family Pisttacidae, Alexandrine Parakeet (*Psittacula eupatria*) is comes under Near Threatened (NT) category of International Union for Conservation of Nature and Natural resources (IUCN), remaining all comes under Least concern (LC) category [15]. A total of seven species of birds were enlisted in Appendix-II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and are Alexandrine Parakeet (*Psittacula eupatria*), Black Kite (*Milvus migrans*), Black-Shouldered Kite (*Elanus caeruleus*), Shikra (*Accipiter badius*), Barn owl (*Tyto alba*), Spotted Owlet (*Athene brama*), Jungle Owlet (*Glaucidium radiatum*) [16].

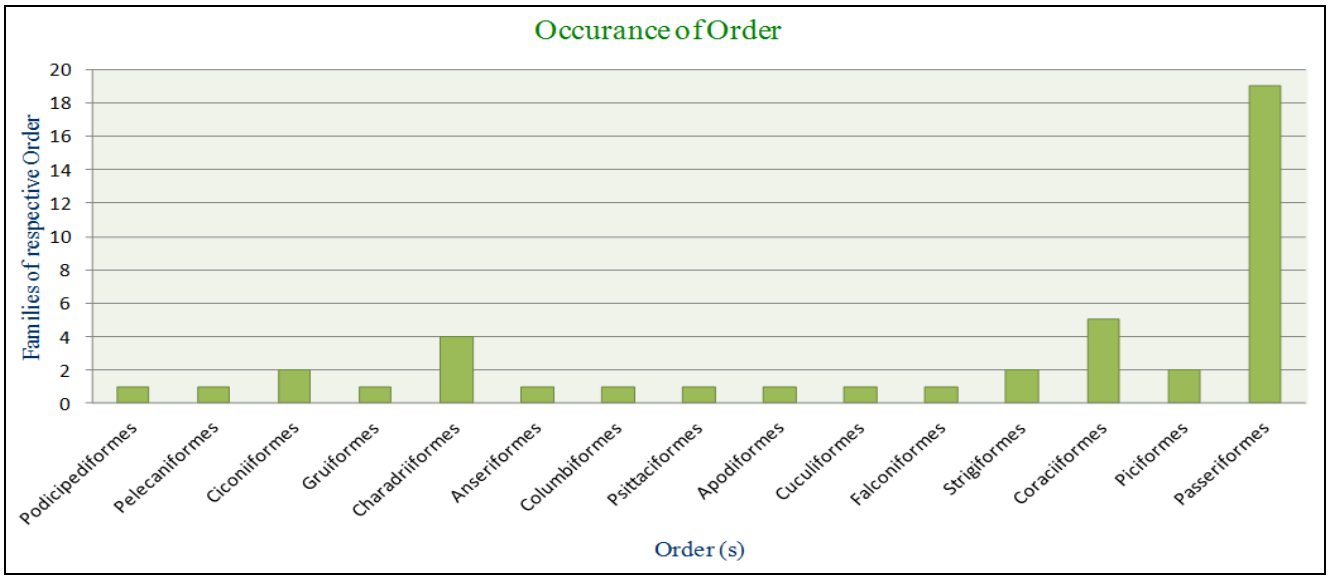

Figure 1. Representing occurrence of order(s) with respect to belonging families of birds.

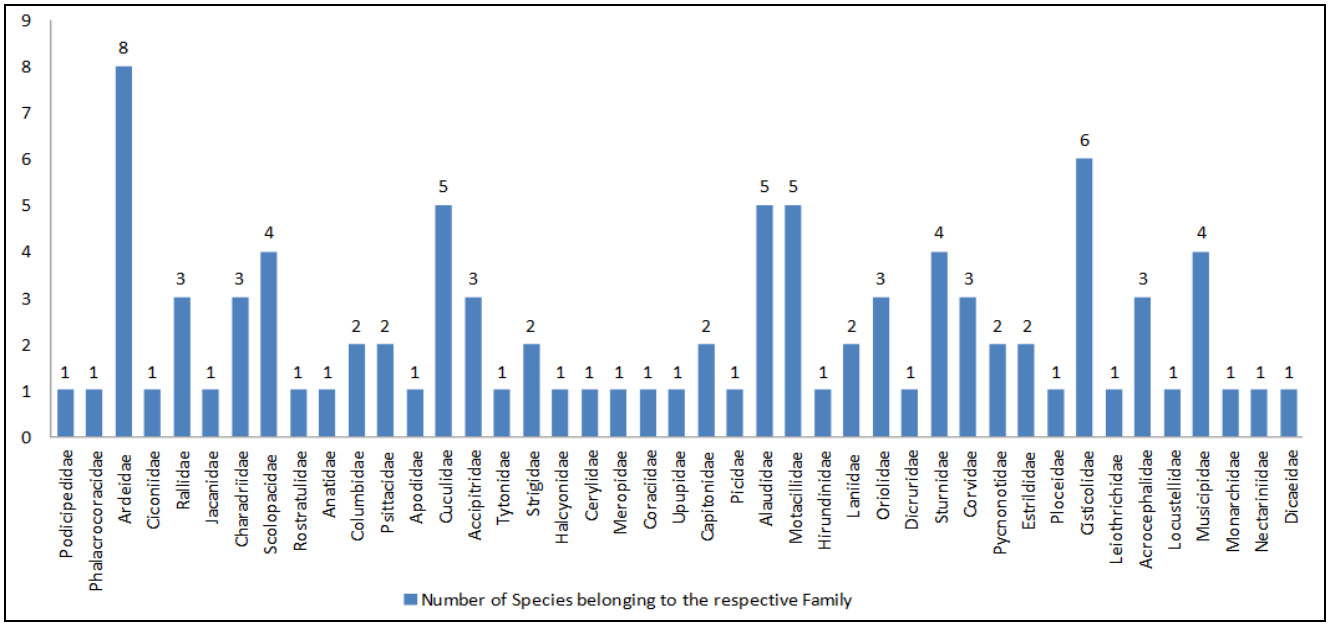

Figure 2. Representing occurrence of family with respect to belonging species of birds.

Table 1. Following are the birds sighted during the study periods, 15 March 2014 - 15 March 2015 at Agronomy field of O.U.A.T, Bhubbaneswar - 751003, India.

Order and Family	Common Name	Scientific Name	Abundance	Status	Protection Status		
					WPA, India	IUCN	CITES
Order: **1.** Podicipediformes **Family:** **1.** Podicipedidae	Little Grebe	*Tachybaptus ruficollis*	U	RS	Sch.IV	LC	NY
Order: **2.** Pelecaniformes **Family:** **2.** Phalacrocoracidae	Little Cormorant	*Phalacrocorax niger*	C	RS	Sch.IV	LC	NY
Order: **3.** Ciconiiformes **Family:** **3.** Ardeidae	Indian Pond Heron	*Ardeola grayii*	C	RS	Sch.IV	LC	NY
	Blackcrowned Night Heron	*Nycticorax nycticorax*	U	RS	Sch.IV	LC	NY
	Purple Heron	*Ardea purpurea*	R	LM	Sch.IV	LC	NY
	Little Egret	*Egretta garzetta*	U	RS	Sch.IV	LC	NY
	Cattle Egret	*Bubulcus ibis*	C	RS	Sch.IV	LC	NY
	Intermediate Egret	*Mesophoyx intermedia*	R	RS	Sch.IV	LC	NY
	Chestnut Bittern	*Ixobrychus cinnamomeus*	U	RS	Sch.IV	LC	NY
	Yellow Bittern	*Ixobrychus sinensis*	R	RS	Sch.IV	LC	NY
Family: **4.** Ciconiidae	Asian Openbill Stork	*Anastomus oscitans*	C	RS	Sch.IV	LC	NY
Order: **4.** Gruiformes **Family:** **5.** Rallidae	Ruddy-breasted Crake	*Porzana fusca*	U	RS	Sch.IV	LC	NY
	White-breasted Waterhen	*Amaurornis phoenicurus*	C	RS	Sch.IV	LC	NY
	Watercock	*Gallicrex cinerea*	U	RS	Sch.IV	LC	NY
Order: **5.** Charadriiformes **Family:** **6.** Jacanidae	Bronzewinged Jacana	*Metopidius indicus*	C	RS	Sch.IV	LC	NY
Family: **7.** Charadriidae	Kentish Plover	*Charadrius alexandrinus*	U	M	Sch.IV	LC	NY
	Yellow-wattled Lapwing	*Vanellus malabaricus*	C	RS	Sch.IV	LC	NY
	Red-wattled Lapwing	*Vanellus indicus*	C	RS	Sch.IV	LC	NY
Family: **8.** Scolopacidae	Wood Sandpiper	*Tringa glareola*	R	M	Sch.IV	LC	NY
	Jack Snipe	*Lymnocryptes minimus*	U	M	Sch.IV	LC	NY
	Common Snipe	*Gallinago gallinago*	U	M	Sch.IV	LC	NY
	Pintailed Snipe	*Gallinago stenura*	U	M	Sch.IV	LC	NY

Order and Family	Common Name	Scientific Name	Abundance	Status	Protection Status		
					WPA, India	IUCN	CITES
Family: **9.** Rostratulidae	Greater Painted-snipe	*Rostratula benghalensis*	R	LM	Sch.IV	LC	NY
Order: **7.** Columbiformes **Family:** **10.** Columbidae	Blue Rock Pigeon	*Columba livia*	C	RS	——	LC	NY
	Spotted Dove	*Streptopelia chinensis*	C	RS	Sch.IV	LC	NY
Order: **8.** Psittaciformes **Family:** **11.** Psittacidae	Rose-ringed Parakeet	*Psittacula krameri*	C	RS	Sch.IV	LC	NY
	Alexandrine Parakeet	*Psittacula eupatria*	U	LM	Sch.IV	NT	APP.II
Order: **9.** Apodiformes **Family:** **12.** Apodidae	House Swift	*Apus affinis*	U	RS	Sch.IV	LC	NY
Order: **10.** Cuculiformes **Family:** **14.** Cuculidae	Greater Coucal	*Centropus sinensis*	C	RS	Sch.IV	LC	NY
	Lesser Coucal	*Centropus bengalensis*	C	RS	Sch.IV	LC	NY
	Asian Koel	*Eudynamys scolopacea*	C	RS	Sch.IV	LC	NY
	Common HawkCuckoo	*Hierococcyx varius*	C	RS	Sch.IV	LC	NY
	Greybellied Cuckoo	*Cacomantis passerinus*	R	LM	Sch.IV	LC	NY
Order: **11.** Falconiformes **Family:** **15.** Accipitridae	Black Kite	*Milvus migrans*	C	RS	Sch.IV	LC	APP.II
	Black-shouldered Kite	*Elanus caeruleus*	U	RS	Sch.IV	LC	APP.II
	Shikra	*Accipiter badius*	C	RS	Sch.IV	LC	APP.II
Order: **12.** Strigiformes **Family:** **16.** Tytonidae	Barn Owl	*Tyto alba*	U	RS	Sch.IV	LC	APP.II
Family: **17.** Strigidae	Spotted Owlet	*Athene brama*	U	RS	Sch.IV	LC	APP.II
	Jungle Owlet	*Glaucidium radiatum*	U	LM	Sch.IV	LC	APP.II
Order: **13.** Coraciiformes **Family:** **18.** Halcyonidae	White-breasted Kingfisher	*Halcyon smyrnensis*	C	RS	Sch.IV	LC	NY
Family: **19.** Cerylidae	Pied Kingfisher	*Ceryle rudis*	U	RS	Sch.IV	LC	NY
Family: **20.** Meropidae	Green Bee-eater	*Merops orientalis*	C	RS	Sch.IV	LC	NY
Family: **21.** Coraciidae	Indian Roller	*Coracias benghalensis*	U	RS	Sch.IV	LC	NY
Family: **22.** Upupidae	Common Hoopoe	*Upupa epops*	U	RS	Sch.IV	LC	NY

Order and Family	Common Name	Scientific Name	Abundance	Status	Protection Status		
					WPA, India	IUCN	CITES
Order: **14.** Piciformes **Family:** **23.** Capitonidae	Coppersmith Barbet	*Megalaima haemacephala*	U	RS	Sch.IV	LC	NY
	Brown-headed Barbet	*Megalaima zeylanica*	C	RS	Sch.IV	LC	NY
Family: **24.** Picidae	Black-rumped Flameback	*Dinopium benghalense*	U	RS	Sch.IV	LC	NY
Order: **15.** Passeriformes **Family:** **25.** Alaudidae	Bengal Bushlark	*Mirafra assamica*	C	RS	Sch.IV	LC	NY
	Ashy-crowned Sparrow Lark	*Eremopterix grisea*	U	RS	Sch.IV	LC	NY
	Singing Bushlark	*Mirafra cantillans*	C	RS	Sch.IV	LC	NY
	Jerdon's Bushlark	*Mirafra affinis*	U	RS	Sch.IV	LC	NY
	Rufous-tailed Lark	*Ammomanes phoenicurus*	R	RS	Sch.IV	LC	NY
Family: **26.** Motacillidae	Paddyfield Pipit	*Anthus rufulus*	C	RS	Sch.IV	LC	NY
	White-browed Wagtail	*Motacilla maderaspatensis*	R	RS	Sch.IV	LC	NY
	White Wagtail	*Motacilla alba*	R	M	Sch.IV	LC	NY
	Yellow Wagtail	*Motacilla flava*	C	M	Sch.IV	LC	NY
	Citrine Wagtail	*Motacilla citreola*	U	M	Sch.IV	LC	NY
Family: **27.** Hirundinidae	Barn Sallow	*Hirundo rustica*	C	RS	Sch.IV	LC	NY
Family: **28.** Laniidae	Brown Shrike	*Lanius cristatus*	C	M	Sch.IV	LC	NY
	Long Tailed Shrike	*Lanius schach*	U	RS	Sch.IV	LC	NY
Family: **29.** Oriolidae	Blackhooded Oriole	*Oriolus xanthornus*	U	RS	Sch.IV	LC	NY
	Black-naped Oriole	*Oriolus chinensis*	R	LM	Sch.IV	LC	NY
	Eurasian Golden Oriole	*Oriolus oriolus*	U	RS	Sch.IV	LC	NY
Family: **30.** Dicruridae	Black Drongo	*Dicrurus macrocercus*	C	RS	Sch.IV	LC	NY
Family: **31.** Sturnidae	Common Myna	*Acridotheres tristis*	C	RS	Sch.IV	LC	NY
	Jungle Myna	*Acridotheres fuscus*	U	RS	Sch.IV	LC	NY
	Asian Pied Starling	*Sturnus contra*	C	RS	Sch.IV	LC	NY
	Brahminy Starling	*Sturnus pagodarum*	R	LM	Sch.IV	LC	NY
Family: **32.** Corvidae	Rufous Treepie	*Dendrocitta vagabunda*	C	RS	Sch.IV	LC	NY
	Common Crow	*Corvus splendens*	C	RS	Sch.V	LC	NY
	Jungle Crow	*Corvus macrorhynchos*	C	RS	Sch.IV	LC	NY

Order and Family	Common Name	Scientific Name	Abundance	Status	Protection Status		
					WPA, India	IUCN	CITES
Family: 33. Pycnonotidae	Redvented Bulbul	*Pycnonotus cafer*	C	RS	Sch.IV	LC	NY
	Redwhiskered Bulbul	*Pycnonotus jocosus*	U	RS	Sch.IV	LC	NY
Family: 34. Estrildidae	Scaly-breasted Munia	*Lonchura punctulata*	C	RS	Sch.IV	LC	NY
	Red Avadavat	*Amandava amandava*	C	RS	Sch.IV	LC	NY
Family: 35. Ploceidae	Baya Weaver	*Ploceus philippinus*	C	RS	Sch.IV	LC	NY
Family: 36. Cisticolidae	Zitting Cisticola	*Cisticola juncidis*	C	RS	Sch.IV	LC	NY
	Plain Prinia	*Prinia inornata*	C	RS	Sch.IV	LC	NY
	Rufous-fronted Prinia	*Prinia buchanani*	C	RS	Sch.IV	LC	NY
	Ashy Prinia	*Prinia socialis*	U	RS	Sch.IV	LC	NY
	Jungle Prinia	*Prinia sylvatica*	U	RS	Sch.IV	LC	NY
	Common Tailorbird	*Orthotomus sutorius*	U	RS	Sch.IV	LC	NY
Family: 37. Leiothrichidae	Jungle Babbler	*Turdoides striatus*	C	RS	Sch.IV	LC	NY
Family: 38. Acrocephalidae	Blyth's Reed Warbler	*Acrocephalus dumetorum*	C	LM	Sch.IV	LC	NY
	Blunt-winged Warbler	*Acrocephalus concinens*	C	LM	Sch.IV	LC	NY
	Clamorous reed Warbler	*Acrocephalus stentoreus*	C	LM	Sch.IV	LC	NY
Family: 39. Locustellidae	Striated Grassbird	*Megalurus palustris*	U	LM	Sch.IV	LC	NY
Family: 40. Musicipidae	Asian Brown Flycatcher	*Muscicapa dauurica*	U	LM	Sch.IV	LC	NY
	Bluethroat	*Luscinia svecica*	U	M	Sch.IV	LC	NY
	Indian Robin	*Saxicoloides fulicata*	R	RS	Sch.IV	LC	NY
	Oriental Magpie Robin	*Copsychus saularis*	C	RS	Sch.IV	LC	NY
Family: 41. Monarchidae	Black-naped Monarch	*Hypothymis azurea*	R	RS	Sch.IV	LC	NY
Family: 42. Nectariniidae	Purple-rumped Sunbird	*Nactarinia zeylonica*	C	RS	Sch.IV	LC	NY
Family: 43. Dicaeidae	Pale-billed Flowerpecker	*Dicaeum erythrorhynchos*	U	RS	Sch.IV	LC	NY

A total of 95 species of birds belongs to 43 Family and 15 Order were recorded during the study.

Table 2. Image of few birds of Agronomy Field of O.U.A.T, Bhubaneswar, 751003, India.

 Jack Snipe	 Common Snipe	 Ruddy-breasted Crake	 Watercock
 White-breasted Waterhen	 Greater Painted-snipe	 Chestnut Bittern	 Yellow Bittern
 Wood Sandpiper	 Blackcrowned Night Heron	 Purple Heron	 Indian Pond Heron
 Cattle Egret	 Bronzewinged Jacana	 Little Cormorant and Little Egret	 Asian Openbill Stork
 Blackhooded Oriole	 Rose-ringed Parakeet	 Green Bee-eater	 Black Drongo
 White Wagtail	 Yellow Wagtail	 Pied Kingfisher	 Whitebreasted Kingfisher

 Redwattled Lapwing	 Yellowwattled Lapwing	 Indian Roller	 Bluethroat
 Brown-headed Barbet	 Black-rumped Flameback	 Shikra	 Black-shouldered Kite
 Red Avadavat	 Scaly-breasted Munia	 Baya Weaver	 Paddy-field Pipit
 Zitting Cisticola	 Ashy-crowned Sparrow Lark	 Common Hawk Cuckoo	 Grey-bellied Cuckoo
 Jungle Babbler	 Asian Brown Flycatcher	 Brown Shrike	 Common Hoopoe
 Spotted Dove	 Blue-rock Pegion	 Common Myna	 Asian-pied Starling

 Jungle Crow	 Common Crow	 Asian Koel	 Black-naped Monarch
 Red-whiskered Bulbul	 Red-vented Bulbul	 Rufous Treepie	 Greater Coucal
 Bengal Bushlark	 Singing Bushlark	 Jerdon's Bushlark	 Rufous-tailed Lark
 Barn Swallow	 Blunt-winged Warbler	 Blyth's Reed Warbler	 Clamorous Reed Warbler
 Spotted Owlet	 Barn Owl	 Plain Prinia	 Rufous-fronted Prinia

4. DISCUSSION

Agro-ecosystems include a large proportion of the world's biodiversity [17]. The highest bird diversity is due to more diversity of plant which provides more choice for the food as well as nesting and breeding sites. The considerable number of trees in fallow land and boundary of agricultural fields accommodates large number of bird population [18]. Birds are key species in an agricultural ecosystem for maintaining the ecological balance [3]. Birds constitute an important component of agro-ecosystems. The dual role of birds in agro-ecosystem is very well known [4, 5]. Birds provi-

ding important ecosystem service like pest control, pollination and seed dispersal [7, 19].

The results obtained from the present study of the agronomy field of O.U.A.T, Bhubaneswar shows the presence of 95 species of birds belonging to 15 orders and 43 families, out of which order Passeriformes was found dominant (44.19% occurrence) with 19 family of bird and order Podicipediformes, Gruiformes, Anseriformes, Columbiformes, Psittaciformes, Apodiformes, Cuculiformes, Falconiformes were found least occurred (2.35% occurrence each) with only 1 family of bird each.

It is presumed that the diversified flora of agronomy field of university provides comfortable shelter, suitable foraging grounds and protection from predation and hostile atmospheric conditions to these birds. Agronomy field of O.U.A.T, Bhubaneswar provides a suitable ground for feeding, roosting, reproduction and nesting to these birds.

This study provides a baseline data of the avian diversity of Agronomy field of O.U.A.T, Bhubaneswar, however present study limits its scope to species diversity of birds, further an attempt should be made to find out the dynamics of recorded bird community in correlation with the environmental condition(s) of agronomy field which can be helpful for better management of the habitat and conservation of its rich avifaunal diversity.

5. CONCLUSION

Although the current study was restricted to a limited period of time (i.e. 15 March 2014 to 15 March 2015), it suggests that the avian fauna of agronomy field of O.U.A.T, Bhubaneswar is sufficiently rich in species diversity. Being the first survey of the area, it provides baseline information for future surveys/studies.

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AUTHORS' CONTRIBUTION

AM: Field data collection, compilation of data, collection of references(s), manuscript preparation, editing; DSC: Field data collection, compilation of data, rechecking of manuscript to minimize faults; AS: Field data collection, rechecking of manuscript to minimize faults; SPP: Field visit and compilation of data, collection of reference(s), final editing and checking of manuscript. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Research Article

Representatives of genus *Sempervivum* in mountain flora of eastern Alps

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ABSTRACT

This article summarizes results from field trip to Passo Stevio in eastern Alps, which purpose was to monitor present *Sempervivum* species in this location. Obtained results were compared with available literature and with results from similar research which was made in bigger scale in 1971-72. Obtained results show that in Eastern Alps can be found all the houseleeks species from 1971 (*S. arachnoideum* L., *S. montanum* L. and *S. tectorum* L.). We also find a new species which is not mentioned in the 1971-72 research (*S. wulfenii* Hoppe ex Mert. et Koch) and we also confirm presence of several natural hybrids between all observed species.

Keywords: *Sempervivum*, Houseleek, Passo Stevio, Population monitoring, Hybrid.

1. INTRODUCTION

Sempervivum is a genus of hardy monocarpic alpine succulents in the family *Crassulaceae*. Genus contains around 50 species and over 3000 named cultivars with a wide range of rosette sizes, forms and colours. Houseleeks are typical succulent plants that need just little bit of soil and full sun position. They are often grown in the gardens where they do well in cracks in dry stone walls, on tuff and in rock gardens.

Research which is presented in this article was done during the botanical trip to Italy. Aim of this trip was to get photographic materials and field data about the mountain flora for educational and experimental work. Some of the obtained data will be preceded and published in form of articles like this; others will be used as basic platforms for future research and cooperation.

2. MATERIALS AND METHODS

Our research took place in Passo Stevio, which is the highest mountain pass of the eastern Alps (Figures 1 and 2). It is situated in the altitude 2757 meters above sea level, in the area of province Sondrio in the north part of Italy. The research area was chosen due to rich plant material presence and easy reachability by car which makes from this area ideal spot for research.

Mapping of the *Sempervivum* species in eastern Alps consisted of four separate trips to the location Passo Stevio in first half of August. During each trip were made several mapping excursions to

the different parts of mountain pass during which was all discovered *Sempervivum* plants were photographed (whole plant, leaf detail, flowers if plant was blooming) and determined. The obtained results were also verified by literature and botany researchers in Czech Republic.

Figs. 1 (up) **and 2** (down). Passo Stevio in eastern Alps – place of research.

3. RESULTS AND DISCUSSION

In mapped area we find all three typical Alpine *Sempervivum* species (*S. arachnoideum* L., *S. montanum* L. and *S. tectorum* L.) and besides them we also found and identified *S. wulfenii* Hoppe ex Mert. et Koch and six interspecies hybrids.

Sempervivum montanum subsp. montanum L. Solons thin. (Figure 3)

Leaves in rosettes are usually 10 x 3 mm (exceptionally can be bigger). Leaves are egg shaped, sharply ended, hairy on both sides. Hairs are short, green and flexible. Flower stem is 50-100 (200) mm high and caries 2-8 (13) flowers, frequently with 11 or 13 petals. Petals are 12-20 x 2 mm big. Flowers are reddish, sometimes yellow flowers occur. Typical signs of subspecies *montanum* is a small rosette (not bigger than 20 mm in diameter), and hairs on the flower stem are longer than those on leaves [1]. It naturally grows in Corsica, Italy, France, Spain (Pyrenees, Alps, north Apennines). It grows in altitudes from 1 700 to 3 000 meters above sea level [2].

Fig. 3. *Sempervivum montanum.*

Sempervivum tectorum L.**,** syn. *Sempervivum assimile* Schott. (Figure 4)

Perennial herb making clumps of compact, dark green, sometime reddish rosettes of fleshy leaves. Sterile rosettes are 30-80 mm in diameter. Rosettes are open, the ends of leaves pointed out from the centre. Leaves are ovoid, 20-40 mm long and 10-15 sometimes even 20 mm wide. The surface is glabrous, just on the edges ciliate. Ends of the leaves are reddish or red-purple. Flower stem grows from the centre of rosette and is 200-500 mm high. After flowering the whole flower stem dies. Inflorescence is made from 5-15 flowers each on short 3 mm long stem. Flowers have 12-16 petals [1]. Seeds prolonged obpyriform or obovoid, flattish, apex rounded, base with or without wing or tail, 0.9-1.2 x 0.4-0.6 mm. Surface longitudinal ribbed, ribs faint crenate, interspaces with indistinct transverse ribs, orange-brown [3]. Naturally grown in France, Italy, Spain (Pyrenees, Alps, Apennines), and in northern parts of Balkans [2].

Fig. 4. *Sempervivum tectorum.*

Fig. 6. *Sempervivum wulfenii.*

Sempervivum arachnoideum L. (Figure 5)

Small species which makes rosettes just around 25 mm in diameter. Leaves have on tips white, spider web like woolly hair. Flowers are pink [1]. Seeds prolonged obovoid, with a small wing on the apex, base narrowed with hilum, 0.7-0.8 x 0.2-0.3 mm. Surface with longitudinal ribs, lustrous, yellowish-brown [3]. This species naturally occurs in France, Italy, Spain (Pyrenees, Alps, and Apennines) [2].

Fig. 5. *Sempervivum arachnoideum.*

Sempervivum wulfenii Hoppe ex Mert. et Koch. (Figure 6)

Slowly growing herbal perennial, making 2-3 rosettes in one growing period which are around 100 mm in diameter. Leaves are grey-green, on the basis purple. Flowers are yellow with purple coloured basis. This species naturally grow in west Alps (Italy, Switzerland and Austria) [2].

Beside these four botanical species, we found several natural hybrids. Natural hybridization is common in the genus *Sempervivum*. Research done in the Pyrenees on 2926 plants shows that species as *S. arachnoideum, S. montanum* and *S. tectorum* often make mutual hybrids [4]. In the research done in 1971 and 1972, out of 224 plants collected in their natural environment, 138 were determined as natural hybrids of *S. tectorum* and 84 as natural hybrids of *S. arachnoideum*.

In the chosen locality, we found and identified hybrids (Figures 7-11) between *S. tectorum* x *S. arachnoideum* (or between *S. wulfenii* x *S. arachnoideum*), hybrids between *S. montanum* x *S. arachnoideum*, hybrids between *S. arachnoideum* x *S. tectorum* (but this could also be hybrid between *S. montanum* x *S. arachnoideum*), hybrids between *S. tectorum* x *S. arachnoideum*, hybrids between *S. tectorum* x *S. montanum.* Exact identification must be deter-mined by AFLP method (Amplified fragment length polymorphism). This method on the basis of DNA fingerprinting is able to determinate relationships between plant species or cultivars on the molecular level.

Significance of the *Sempervivum* family is in their medicinal usage. For example *Sempervivum tectorum* L. has a long tradition of using in folk medicine in Alpine regions and its healing properties are similar to the properties of aloe plant. *S. tectorum* L. is used as a cure to shingles, dysentery and headaches. It was used with the burns, scald wounds, stings and skin diseases and also for the ear and eye wounds. It should heal corns and warts. The leaves of houseleeks are chewed as a remedy against the toothache. Nowadays, it is used

for the burn wounds treatment or for the insect stings [5, 6]. The researches of several authors within the years 1993-2003 mentioned positive effect of *Sempervivum tectorum* L. on healing of liver, its usage as a painkiller and also its stabilizing effect to the cell membranes [7-9].

In literature it is mentioned that in the eastern Alps are native three species of houseleeks *S. arachnoideum* L., *S. montanum* L. and *S. tectorum* L. The Alpian houseleeks grow in xerothermal sites and can survive 7-8 months of snow. Main growing period of these plants is in spring (V-VI) and flowering period in summer (VII-IX). *S. montanum* L. occurs in the altitudes from 1800 to 2300 meters above sea level, Barun even mentions an altitude of 3250 meters. *Sempervivum arachnoideum* L. and *Sempervivum tectorum* L. are common in habitats in altitudes from 600 to 2800 meters [10]. This shows that houseleeks are plants extremely resistant to the climatic conditions, which is proved by many authors and their researches.

Fig. 7. *Sempervivum arachnoideum x S. tectorum (S. montanum x S. arachnoideum).*

Fig. 8. *Sempervivum tectorum x S. arachnoideum.*

Fig. 9. *Sempervivum montanum x S.arachnoideum.*

Fig. 10. *Sempervivum tectorum x S. montanum.*

Fig. 11. *Sempervivum tectorum x S. arachnoideum (S. wulfenii x S. arachnoideum).*

Larcher et al. in 2010 examined resistance of high mountains flora to the extreme temperatures. Their research was based on the assumption that mountain plants can during summer survive a short term swing which is higher than average temperatures in the summer months. In the clear and

windless days, the leaf rosette in plants can be oriented to the south warm to the temperature 40 or even 50 °C. The experiment proved that *Sempervivum arachnoideum* L. and *S. tectorum* L. have the ability to survive, for the short period of time, temperatures around 62-64 °C [10]. This research was followed by Zaharia by experiment, in which they examine the resistance of *S. montanum* L. and *S. tectorum* L. to the extreme conditions. Rosettes of these two species were exposed to a temperature of 50 °C, light intensity of 90000 lux together with dry soil conditions. The results of experiment show that both examined species are extremely resistant [11]. Also resistance to the low temperatures is significant for the *Sempervivum* plants. When the leaves of *S. montanum* were exposed to the low temperatures during the summer growing period, they were damaged by a temperature -5 °C, at temperatures from -6 to -7 °C, half of the rosette died, by the temperatures from -8 to -9 °C the whole rosettes died. But when the plants were treated with zero temperatures for three days, the resistance of leaf rosettes increased by 5 degrees (the leaves started to die at -13 °C), when the cold period was even longer the lethal temperature was lowered again, to the -25 °C [10].

In the end we can say that the area of Passo Stevio is, from the botanical point of view, a very interesting locality. Good accessibility by car makes it a perspective locality for a research of the alpine flora. Our research confirms information from the literature about the species' occurrence of genus *Sempervivum* plants. We confirm presence of all the three most common Alpine houseleeks and also found six natural hybrids, whose exact determination must be confirmed by molecular methods.

AUTHORS' CONTRIBUTION

KK: leads the information research, works with scientific databases and was completing part Results and Discussion. PK: was in charge in field research, photo documentation and writing of all the other parts of the article. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Current Life Sciences

Research Article

Management of white rot of onion using composts and *Trichoderma harzianum*

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ABSTRACT

Onion white rot (OWR) is the most serious disease of *Allium cepa* L. caused by *Sclerotium cepivorum* Berk, it causes an economical loss in the main production of onion in Egypt and around the world. In this study the effect of three types of compost and bioagent (*Trichoderma harzianum*) in controlling white rot disease of onion under greenhouse conditions was studied. All tested compost extract effect on number of sclerotia in *vitro* at all tested concentrations, while *T. harzianum* gave over growth. The tested types of compost was recommended by El-Nile Company (ENC), compost 2 contains fresh cow manure, poultry manure (CCP), while compost 3 was mad palm leaves (CPL). Compost applied as post with *Sclerotium cepivorum*. *T. harzianum* gave the lowest percentage of infection (26.66) followed by lowest compost 2 while, compost 3 was the lowest effect on the disease incidence occurrence. Onion white rot (OWR) considers a devastating disease of *Allium spp*. caused by the *S. cepivorum* which is consided to be an important economical loss in the main production area of winter onion (*Allium cepa* L.) in Egypt and all over the world. The use of compost 1 at the rate of 15% W/W (5 g/kg) reduced disease severity compared to control. The use of *Trichoderma harzianum* at *Sclerotium cepivorum* against as a bioagent at the rate of 5% W/W of the soil reduced disease incidence compared to control.

Keywords: Onion white rot; *Sclerotium cepivorum*; *Trichoderma harzianum*; Compost; Biological Control.

1. INTRODUCTION

Allium white rot (OWR) is a serious disease of onion and other *Allium spp*. that has a significant economic impact on crop yields. The disease is caused by the soil borne pathogen *Sclerotium cepivorum* Berk which attacks the root system of host plants, resulting in either death before or post harvest. The pathogen survives in the soil as sclerotia and may remain dormant in this state in the absence of the host for more than 20 years. Sclerotia are stimulated to germinate by volatile thiols and sulphide released by soil micro-organisms from alkenyl systenine sulfoxides secreted.

In Egypt white rot disease was observed in 1929 [1]. In Upper Egypt, in heavily naturally infected soil, percentage of infection reaches about 100% [2]. The disease has been found in every country where onions and other *Allium spp.* are cultivated [3]. The disease severely reduced onion production in Upper Egypt where onion cultivation is concentrated in Qena, Sohag, Assiut, Menia, Beni-Suef and Fayoum Governorates. Data revealed that onion white is responsible for crop losses of 5-50%, or might be higher, due to the high rot levels of sclerotia of *S. cepivorum* in soils (range measured 5-232/kg soil). A number of alternatives to fungicides have been investigated to attempt to control the disease. These methods include solarization, the use of biological control agents (BCAS), and sclerotia germination stimulants such as diallyl disulphide (DADS), but there have been problems with inconsistent control. Composts have been used to suppress soil borne pathogens. Loss of the disease suppressive effect of composts following sterilization indicates that the suppressive effect was predominantly biological rather than physical or chemical in nature. It is well known that many micro-organisms have antibiotic producing power to secrete a lethal principle toxic to some fungi.

Trichoderma harzianum has been identified as a promising biocontrol agent of onion white rot disease. Under low to medium disease pressure, the biocontrol fungus gave good control of the disease (60-70%) when applied as a soil additive at planting time [4]. However, under high disease pressure, efficacy of the biocontrol agent declined (30%), necessitating a combination of measures to obtain adequate disease control.

The objectives of this work were to determine the ability of same composts and amending composts with *T. harzianum* to control white rot disease of onion.

2. MATERIALS AND METHODS

2.1. Isolation and identification of the casual pathogen

Onion plants showing white rot symptoms were collected from different localities of Assiut Governorate. Diseased bulbs were washed with tap water, cut into small pieces and surfaces sterilized by immersing in 1% sodium hypochloride solution for two minutes, then rinsed several times in sterile water. Disinfested samples were dried in between sterile filter papers, then plated on potato-dextrose-agar (PDA) medium, containing 40 mg strepophenicol/100 ml, then incubated at 20^{o}C. After 4-5 days incubation, the developed fungal was purified by using hyphal tip isolation techniques [5]. Identification of the fungal isolates was carried out according to Ciements and Shear [6]. The obtained pure cultures were kept at 5^{o}C on PDA slants for further studies.

2.2. Pathogenicity tests

Pathogenicity of *Sclerotium cepivorum* isolates were carried out on Giza 6 onion cultivar under greenhouse conditions during 2010/2011 season. Seedling onion used in this experiment was sterilized by dipping in 10% Chlorox solution for 3 minutes, then rinsed for several times in sterile water. Soil and pots (25 cm in diameter) were sterilized by 5 % formalin solution and left to dry for two weeks.

Inocula (12 isolates) of the pathogens were prepared by inoculating sterilized milk bottles 500 ml, containing sand-corn meal medium (20 kg coarse sand, 1 kg ground maize + 4 liter of water) with the tested fungal isolates, then incubated at 20^{o}C for 30 days [7]. Inoculum of each isolate was added to the potted soil at rate of 2 sclerotia/1g soil and mixed well with soil for each pot. Each pot was sown with 5 seedling onion and 3 pots were used as replicates. Pots containing non infested soil served as control. After 6 months from sowing, plant per pot was uprooted, examined for white rot symptoms and disease severity was rated to the following scale:

0 = healthy
1 = bulb covered with mycelium but not rotted
2 = 1-25% of the bulb rotted
3 = 25-50 % of the bulb rotted
4 = 50-75% of the bulb rotted
5 = 75-100 % of the bulb rotted [8].

Disease was scores were converted into percentage as follows:

$$\text{Disease severity (\%)} = \frac{\text{Total points score} \times 100}{\text{Total number of bulbs} \times \text{highest score}}$$

2.3. Evaluation the suppressive effect of certain compost on the incidence of white rot disease of onion

Three different composts in (Table 1) were used through this study. The compost 1 manufactured by El-Nile company (CEN) is commercially available and certified for us as organic manure to improve productivity of some crops. This compost used through our study in fertilizing onion plants with specific dose (5 ton/Fadden) equivalent to 25 g inch pot experiment. The compost 2 is manufactured by using fresh caw manure, poultry manure and urea as 2% on dry weigh basis. Animal manures and urea were applied as a nitrogen source to speed up composition and to improve compost quality. Completion of composting was determined by the pleasant odors reduction of the produced heat, the change into dark brown color and the crumbling texture type of the mixture specific dose (2 ton/ Fadden). The compost 3 manufactured by palm leaves and animal manure with specific dose (2 ton/ Fadden), equal 10g/per pot (5 kg soil).

Table 1. Chemical analysis of three different composts used this study.

Nutrient element G/kg		Compost 1	Compost 2	Compost 3
C	%	323.24	19.7	15.64
N		14.88	1.4	0.90
$P_2 O_5$		10.02	0.75	0.79
K_2O		12. 80	1.0	0.94
PH		8.0	8.38	7.0
Fe (ppm)		None	1850	None
Zn (ppm)		None	225	None
Mn (ppm)		None	50	None
C/N (%)		None	14.1	17.38
RH (%)		56	30	27

2.4. Effect of compost and *T. harzianum* on total sclerotia of *S. cepivorum* in *vitro*

Laboratory work were directed to study the effect of watery extracts three compost and *T. harzianum* on total percent germination of *Sclerotium cepivorum* in *vitro.* All composts were tested at concentration of 2.5, 5, 10 and 15%. The water extracts of composted were prepared by 1 g of compost was placed in 99 ml of water. The mixture was blended for 30 sec. at high speed in blender and sterilized by microfiltered (0.2 mm). Petri-dishes (9 cm diameter) containing PDA medium were inoculated in the centre with discs (5 mm in diameter) of *Sclerotium cepivorum* taken, from 7 days old culture. For studying effect *T. harzianum* on growth sclerotia plates were inoculated with equal disks at the centre of plate. Two disks was also placed at opposite sides at the periphery of plate at the same distance from the disk of antagonist. Control mixed with sterilized water. All plates were incubated in the dark at 24^{o}C and incubation for 14 days. At the control was full the sclerotia recorded in treatments (5 mm in diameter).

2.5. Effect of three different composts and *Trichoderma harzianum* on the incidence of white rot diseases

The highly pathogen isolate of *S. cepevorium* was selected to be used in pot experiments and under greenhouse conditions. The experiment was conducted during 2011/2012 and 2012/2013 seasons using three compost, the nutrient element content were in Table 2. Sterilized pots (30 cm in diameter) were filled with sterilized soil. Inoculum of isolate was prepared and mixed as previously described. The three Compost were applied and mixed with sterilized soil at rate 15% w/w and antagonists *Trichoderma harzianum,* were added at rate of 5% w/w soil weight. After seven days from the previously treatment pots were planted with 5 seedling onion Giza 6 cultivar. Three replicate were used and free from composts or *Trichoderma* used as control. Disease severity was recorded after 115 days from sowing.

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of the casual pathogen

White rot (WR), which is caused by the soil borne pathogen *Sclerotium cepivorum* Berk, is one of the most important diseases wherever edible *Allium cepa* crops are grown and is an economically

limiting factor in production areas around the world [9]. Twelve isolated of *S. cepivorum* were isolates from different localities of Assiut Governorate in Egypt. All obtained fungal isolates proved to be able to infect onion plants causing white rot symptoms.

Data in Table 2 indicated that isolate No. 10 showed the highest percentage of infection. No. 10 was selected to be the tested pathogen this studied. The variation in pathogenic capability may be due to the presence of genetic differences among the fungal isolates. These results are in agreement with other studies [1-3].

Table 2. Pathogenicity tests of *Sclerotium cepivorum* isolates on Giza 6 onion cultivar under greenhouse conditions.

Isolates number	Disease severity plants*	Source of isolates
1	45.8	Alfatth
2	50	Alfatth
3	85.5	Abnoub
4	33.33	Abnoub
5	53.47	Manflout
6	88.5	Assiut
7	52.08	Alfatth
8	43.33	Manflout
9	26.66	Assiut
10	91.33	Assiut
11	37.5	Sahil-Selim
12	36.66	Sahil-Selim
Control	0.00	

L.S.D. at 5% = 15.64

* Data recorded after 3 months from sowing date

3.2. Effect of compost and *T. harzianum* on total of sclerotia of *S. cepivorum* in *vitro*

The effect of three composts and *Trichoderma harzianum* on total of sclerotia of *Sclerotium cepivorum* in *vitro* by used four different concentrations are shown in Table 3. All concentration significantly reduced the number of sclerotia. However, increasing concentration, generally increased percent reduction of sclerotia number. The highest reduction was pronounced 15% concentration, while *Trichoderma harzianum* gave overgrowth. Effect sterilized compost extracts was probably attributed to the presence of adequate amounts of essential nutrients derived from composts [10, 11]. In the present study, *T. harzianum* showed mycoparasitism on S. *cepivorum in vitro.* McLean [12] found that *in vitro* assays were conducted to determine possible modes of action of *T. harzianum* C52 significantly reduced the mycelial growth of *S. cepivorum* colonies and prevented three of the four *S. cepivorum* isolates tested from producing sclerotia. Such result has been widely reported [13-15].

Table 3. Effect of compost concentration on total of sclerotia of *Sclerotium cepivorum.*

Concentration (v/v)	Percent of germination		
	Compost 1	Compost 2	Compost 3
2.5	66	60	70
5.0	45	35	50
10.0	25	20	30
15.0	3	2	5
Control	92	92	92

L.S.D. at 5%: Compost (A) = 3.02, Compost (B) = 5.67.

3.3. Evaluation the suppressive effect of certain compost on the incidence of white rot disease of onion

Under greenhouse condition were tested three composts on incidence of white rot diseases in 2011/2012 and 2012/2013 seasons. Data in Table 4 indicate that all tested treatments (compost 1, 2 and 3 and *T. harzianum*) reduced disease severity percent. Generally, all treatments significantly reduced the infection percent when compared to the control at both seasons 2011/2012 and 2012/2013. *T. harzianum* showed the lowest percentage of infection in both season (25; 26.66) followed by compost 2 while, compost 3 showed the lowest percentage of infection.

Sclerotia viability reduced in all three compost, but white rot was suppressed by compost 2. This indicates that the compost had an influence on white rot that was independent of their effect on sclerotia viability. A possible factor is soil pH, in compost 2 and compost1 which had a higher pH than the other compost 3. Increased pH of soils, suppressed OWR. This indicates that slightly alkaline conditions (pH 8.1-8.4) were suppressive to OWR, whereas higher or lower pH values were conducive. According to studies of Coley-Smith

[16] and Rondomanski [17], compost amendment slightly increased soil pH, probably due to mineralisation of compost organic nitrogen into ammonium salts [18]. The presence of the composted in soil increased the rate at which the mixture dried out in the pots, probably due to increased drainage. Sclerotia of *S. cepivorum* which are dried out for short periods and then remoistened in soil leak nutrients [19]. Also microelement Zinc effect on *Fusarium* wilt of safflower and reduced the percentage of infected plants [20].

Table 4. Effect of three compost types and *Trichoderma harzianum* on *Sclerotium cepivorum* under greenhouse conditions.

Treatments	Disease severity plants (%)	
	2011/2012	**2012/2013**
Compost 1	52.86	51.91
Compost 2	47.83	47.36
Compost 3	54.95	55.8
T. harzianum	25	26.66
Control	91	91.33

L.S.D. at 5 %: = 11.2, = 6.31.

Micro-organisms in close proximity to the sclerotia use these nutrients to colonies and ultimately degrade the sclerotia [19]. Also found that reducing the soil moisture level for 7 days and then remoistening the soil reduced the survival of sclerotia of *S. cepivorum* [21]. Soil organic amendments serve as a source of nutrients that result in an increase in the soil microbial population, which may have an effect on disease suppression [22]. Decrease in the survival of the sclerotia in this study in the compost over time may have been due to the development of an antagonistic microbial population in soils. The use of organic soil amendments in combination with known antagonists of *S. cepivorum* [23] requires further investigation.

The optimum temperature for sclerotial germination of *S. cepivorum* is c. 15^{o}C [24]. The difference in temperature between the glasshouse and field may therefore explain the greater reduction in viability of sclerotia retrieved from the glasshouse pots compared with the field.

Several mechanisms, by which *Trichoderma spp.* influences plant development have been suggested, such as production of growth hormones, solubilization of insoluble minor nutrients in soil [25] and increased uptake and translocation of less-available minerals [26, 27]. Uptake of certain minerals, such as P and N, is of key importance considering their role in plant growth [28, 29]. Promotion of growth and yield by *Trichoderma spp*. may be a result of increased root area, allowing the roots to explore larger volumes of soil and by this to access nutrients, increased solubility of insoluble compounds as well as increased availability of micronutrients [25, 30]. However, initially *Trichoderma* must be able to establish an interaction with the root system. The ability of a *Trichoderma* species to colonize the root system of a plant depends also on the plant species.

Positive effects of *T. harzianum* on plant growth depend on the rhizosphere conditions and the ability of the fungus to survive and develop in it [27]. Availability of water in the soil may play an important role in facilitating establishment and effectiveness of *Trichoderma* in the soil [31]. Among the positive effects of *Trichoderma* on different plants that have been noted over the past five to ten years in studies conducted by other authors [32].

T. harzianum ability in colonization of interior roots and presence of exogenous IAA in the rhizosphere, enhanced root development and increased root hair formation and could be stimulus in nutrient transfer from soil to root exactly in soils relatively poor in nutrients [27, 33]. The results reported by Vinale et al. [34] clearly indicated that some *Trichoderma* secondary metabolites are directly involved in the *Trichoderma*-plant interactions, and particularly that the compound 6PP may be considered to act as an auxin-like compound and/or may act as an auxin inducer. *T. harzianum* C52 was unable to control onion white rot disease under high disease pressure (87%). The results of these disease control trials suggested that *T. harzianum* may be better applied as part of an integrated disease management program rather than alone [12].

4. CONCLUSIONS

It was concluded that different concentrations of compost have an obvious inhibitory effect on the number of sclerotia of *Sclerotium cepivorum* but *T. harzianum* gave overgrowth in *vitro*. Biocontrol

of onion disease by *T. harzianum* was the best used method, and compost 2 respectively in greenhouse conditions.

AUTHORS' CONTRIBUTION

HAMA and NGA participated in the planning of their experiment and carried out it, analyzed the data and wrote the manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Research Article

Factors affecting the spatial distribution of plant species in Nile islands of mid Egypt

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ABSTRACT

Altogether 152 species of vascular plant species were recorded, belonged to 49 families and 117 genera in 15 islands of the Nile at mid Egypt. The distribution of species showed high variability, the highest was 107 species in canal banks followed by the waste lands, 99 species; while the lowest was 27 species in onion farmlands. The application of Twinspan and Canonical Correspondence Analysis (CCA) resulted in the division of the monitored islands into 2 clear sets, submerged temporary and unsubmerged permanent islands that were subdivided into 4 subgroups. The correlation of these subgroups to the soil variables prevailing in the area of study was discussed. The islands of group A were highly correlated with sand and phosphates; group B was correlated with sand, nitrogen, organic matter and phosphates; while group C was affected by pH, electric conductivity, magnesium, and bicarbonates but islands of group D were affected by silt and organic matter. The interset correlation of the soil variables showed that CCA axis 1 was highly positively correlated with the electric conductivity (0.6465) and highly negatively (-0.4574) correlated with sand content of the soil. While, CCA axis 2 was highly positively (0.2557) correlated with the electric conductivity and highly negatively (-0.3891) correlated with organic matter. The ten different habitats-farmlands were divided into 4 different groups: Group A, comprised the orchards; group B, comprised the winter crops; the waste lands and canal banks were in group D and corn as the only monitored summer crop in Group C.

Keywords: Flora, Agro ecosystem, Weed-soil relationships, Statistical analyses, Sociological ranges.

1. INTRODUCTION

Egypt is an oasis created by the river in a region that was otherwise barren desert [1]. And today it renders fertile the only parts of Egypt which are productive of wealth; called the Nile valley and the delta of the Nile [2]. According to Rzoska [3], the Nile valley began to from 5.5 million years ago, during the Miocene period.

Before reaching the Mediterranean Sea, the Nile flows for about 6700 km through ten countries in north-eastern Africa [4]. Meister proposed that the source of the Nile is the Kagara River in Burundi [5]. It flows through lakes Victoria, Lyoga,

Albert and Nasser, and its mouth is in Egypt at Mediterranean Sea. In 2008 El-Abassery and Hassan [6] cited that the length of the Nile in Egypt is about 1200 km from the southern border at Aswan to the Mediterranean coast. The Nile system has been subjected to large scale schemes of river control, as barrages and dams which have been built across the Nile. These have segmented the natural hydro biological system with undoubted effect on biota [7]. The construction of Aswan High Dam during 1960s affected the river morphology including islands formation and types [8].

El-Abassery and Hassan [6] cited that all of the islands are wetlands which are considered as a reservoir for the biodiversity and have special importance in the life of migratory birds, also all islands are surrounded by aquatic plants like *Phragmites* and Acacia trees (*Acacia nilotica*) which are considered good habitat for birds.

The earliest phytosociological study on weed in Egypt carried out by Tadros and Atta [9] who described the communities of rain fed barely fields in the western Mediterranean coastal region. Then, many studies have been carried out in the Nile region but most of them are floristic [10-14].

Hassib [15] cited that the flora of the Nile in Egypt comprises about 534 species (excluding algae), about 25% of the flora of the country. Many studies of the flora of the Nile have been carried out, particularly after the establishment of Aswan High Dam [16-37]. According to these studies, the vascular fresh water weed flora of Egypt includes 87 species of flowering plants in 45 genera representing 25 families: 12 are Dicotyledons and 13 are Monocotyledons in addition to 3 Pteridophytes's species (*Azolla filiculoides*, *Marsilea egyptiaca* and *M. capensis*).

El-Abassery and Hassan [6] proposed that there are some 500 islands in the main stream of the Nile and its Rosetta and Damietta branches. In addition, El-Hadidi and Hosni [38] indicated that these islands have great diversity in origin, size and structure; 144 of them have been designated in 1996 as protected areas and spread over 16 governorates; 27 of these protected islands are located at Aswan [39]. Moustafa [40] showed that Beni-Suef governorate has 46 islands that differ in length, width and space from each other with a total space of about 4500.12 Fadden (Fadden = c. 4200 m²).

Few studies were concerned with the plant life-forms of the riverian islands in the upper Nile valley in Egypt. Springuel [41] studied the natural vegetation of the islands of the first Cataract at Aswan, and El-Khatib [42] defined the current and past vegetation types of Kraman islands in the Nile at Sohag governorate. Mohamed and Hassan [43] described the plant life-forms of sedimentary islands in Minya governorate, while Hamada [44] studied the plant life-forms of seven islands at Aswan governorate. Recently Hamed et al. [45] worked on some riverian islands at Qena governorate. Also Abd El-Ghani [46] stated that the studies on the vegetation of aquatic ecosystems on the Nile islands have received little attention.

In a study of the species migration route in Nile islands in the same area, Amer et al. [47] confirmed that the most species rich families were Poaceae, Asteraceae, Fabaceae and Brassicaceae that made up almost 44.4% of the total flora. On the other hand, 28 families were very poor and represented by only one species each. Also, the life forms of the collected flora were dominated by the therophytes, 86 species with average of c. 57% followed by the hydrophytes (17.8%) and geophytes (7.2%). Chaemophytes and hemicryptophytes were equally represented by 7 species each, or about 4.6% of the total flora of the area. Nano-phanerophytes were represented by relatively considerable number of species (9 species, c. 6%), in comparison to meso- and microphanerophytes that were represented by only one species each.

The analyses of spatial variation in multi-species weed communities with environmental factors could be useful as a tool to develop a sustainable long-term weed control and soil management strategy [48]. At the same soil condition, some species thrive well [49] in addition the probability of finding these species growing together might be great, even though other factors also influence their abundance, as climate, ability for competition, seed production, capacity and geographic distribution. Because of their ecological requirements, weeds of Egyptian croplands differ from season to another. Several studies [50, 51] indicate that weeds can be grouped into 3 main categories according to their seasonal performance: 1) summer weeds, which are mostly restricted to the warmer months of the year; 2) winter weeds, which

are more mostly restricted to the cooler months of the year; and 3) all-year weeds, which are present and active biologically throughout the year.

Application of numerical methods, as correlation and cluster analyses, and multivariate techniques such as canonical correspondence analysis, can be useful to understand the relationships between weed species and crops. The application of multivariate analysis techniques in weed studies was conducted in Egypt by several researchers in the Nile delta [28, 52-54]; in southern Sinai [55] and in Nile valley [56].

The aims of this work are: a) studying the floristic composition of representative Nile islands in mid Egypt, b) studying the factors that affect the spatial distribution of the flora in the Nile islands, c) studying the affinity between weeds and crops in the selected islands, d) to assess the influence of some environmental factors on weed species composition and distribution.

2. MATERIALS AND METHODS

The studied area, Beni-Suef Governorate, is located at road distance of about 90 km south of Cairo and consists of seven districts namely from south to north: El-Fashn, Somosta, Ehnasya, Beba, Beni-Suef, Nasser and El-Wasta (Fig. 1). It embraces three main terrestrial habitats; desert, fallow land and cultivated land. The rainy season stretches from November to April with a total annual rainfall of about 7.8 mm. The mean temperature values varies between 12.2°C and 29.1°C in January and July respectively. The mean relative humidity ranges between 35% in May and 57% in December [57].

2.1. Selected islands

Fifteen out of 46, the total number of Nile islands at Beni-Suef Governorate, were subjected to almost seasonal visits between 2009 and 2013 (Fig. 1). The selected 15 islands were classified into 7 inhabited permanent (unsubmerged) islands that were cultivated throughout the whole year, another 7 uninhabited submerged islands that were cultivated only in months of low Nile water level from November to June in most years and 1 uninhabited submerged and uncultivated island throughout the year. The islands that were covered with water in summer months, from July to October, are called temporary or submerged islands.

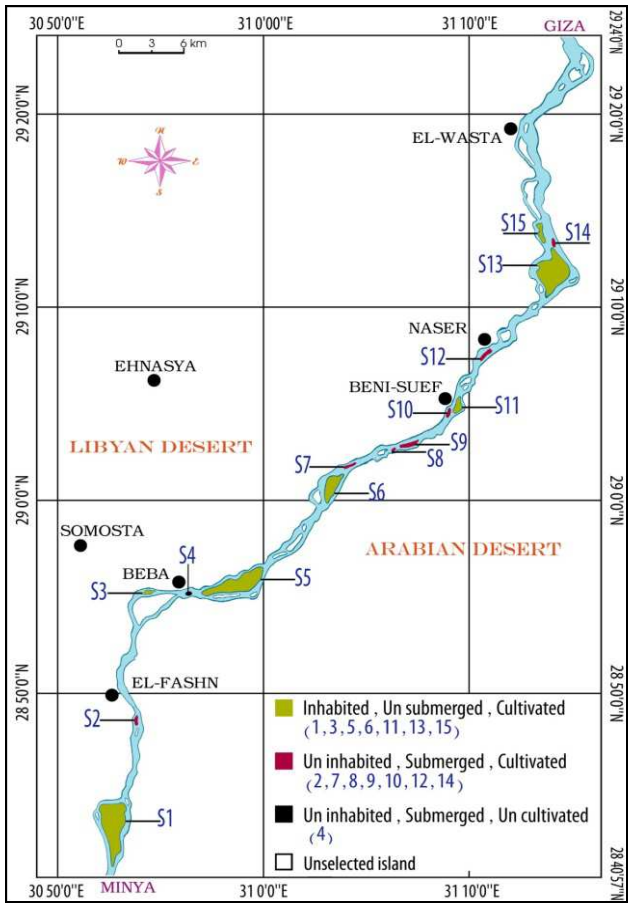

Fig. 1. Investigated islands along the Nile River at Beni-Suef governorate. S1 - Zawet El gedamy, S2 - Awlad Shaker, S3 - El-Foqaey, S4 - Al Shoqr, S5 - Beba, S6 - Sannor, S7 - Bani-Soliman, S8 - Tall El-Nayrouze, S9 - Beni-Suef, S10 - Abu Selem, S11 - El-Alalma, S12 - Mansheyat Al-Sherka, S13 - Al Koraymate, S14 - BaniHeder, S15 - Baget Saleh.

2.2. Soil sampling

To give an overview of the effect of the soil variables on the distribution of the flora in the area, fifteen samples were collected, each represented an island (site). Each sample was a composite sample collected from the monitored stands in each site. These were collected from each stand at a depth of 5-50 cm. The samples of the stands of each site were then pooled, forming one composite sample, air-dried, passed through a 2 mm. sieve to remove gravel and debris, and then packed in paper thoroughly mixed and bags ready for physical and chemical analysis. Three replicates were analyzed

for each sample measurement. Fifteen soil variables (physical and chemical) were investigated namely: soil reaction (pH), electric conductivity (EC), organic matter (OM), sand, silt, clay, bicarbonates (HCO^{3-}), chloride (Cl^{-}), sulfates (SO_4^{2-}), nitrates (NO^{3-}), phosphates (PO_4^{3-}), Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}.

2.3. The selected species

The specimens were collected, identified and checked at Cairo University herbarium (CAI). The identification and nomenclature are mainly based on [17, 58-63]. Voucher specimens were kept at Cairo herbarium (CAI).

2.4. Floristic sampling

Seventy seven stands have been chosen to cover the variations of habitats in the selected islands. The stands were: 15 canal banks, 11 waste lands and 51 cultivated lands. The latter were subdivided into 42 crop farmlands: 13 wheat, 14 clover, 7 corn, 4 onion and 4 lupin stands; in addition to 9 orchards: 2 mango, 5 banana and 2 orange stands. The size of the stand was 20 × 100 m, which approximates the minimal area of weed associations in the study area. Such size was adopted by some other authors [55, 56, 64, 65].

Frequency of occurrence (f%) of species was calculated as the number of stands where the species was recorded divided by the total number of stands at each site (island). The presence performance (P%) of each species was then calculated as the number of stands where the species was recorded divided by the total number of stands.

The frequency of occurrence of the recorded species were organized into five categories: 1) Species recorded in 13-15 sites; 2) Species recorded in 10-12 sites; 3) Species recorded in 7-9 sites; 4) Species recorded in 4-6 sites and 5) Species recorded in 1-3 sites.

2.5. Multivariate analyses of the data

Both classification and ordination techniques were employed. Unicates of the total flora were estimated from the data set to avoid noise and summarize redundancy [66]. A floristic presence/ absence data matrix consists of 15 sites (islands) and identified 152 species was classified by Two-Way Indicator Species Analysis (TWINSPAN) using the default setting of the computer program CAP for windows (Community Analysis Package, version 1.2). The sites were ordered first by divisive hierarchical clustering, and then the species were clustered based on the classification of sites. An ordered two-way table that expresses succinctly the relationships of the samples and species within the data set was constructed [67, 68]. To assure the robustness of the resultant classification matrix with minimum variance (also called Ward's method) as agglomeration criterion [69] of Multi-Variate Statistical Package for windows (MVSP) version 3.13g [70] was used. A dendrogram was elaborated according to TWINSPAN analysis.

The basic goal of ordination is to summarize the community patterns, and to compare these with the environmental information. In this study, the default option of the computer program CANOCO software version 4.51 [71] was used for ordination. The direct gradient analysis was undertaken using Canonical Correspondence Analysis CCA [72].

Preliminary analysis were made by applying the default options of the DCA [73] in the CANOCO program, to check the magnitude of change in species composition along the first ordination axis (i.e., gradient length in standard deviation units). DCA estimated the compositional gradient in the floristic data of the present study to be more than 4 S.D. units for most subset analysis, thus Canonical Correspondence Analysis (CCA) is the appropriate ordination method to perform direct gradient analysis [74].

A Monte Carlo permutation test (499 permutations; [75]) was used to test for significance of the eigenvalues of the first canonical axis. The use of canonical coefficients in determining the significance of environmental variable is undesirable because they can be unstable. Inter-set correlations from the CCA's were therefore used to assess the importance of the environmental variables. All data variables were assessed for normality (SPSS for window version 20) prior to the CCA analysis, and appropriate transformations were performed when necessary to improve normality according to [76].

The TWINSPAN floristic groups were subjected to ANOVA (one-way analysis of variance) based on soil variables to find out whether there

were significant variations among groups. The similarities between each pair of the studied ten habitats, 3 orchards, 5 croplands, canal banks and waste lands were estimated by using the linear correlation coefficients. Application of the cluster analysis to the presence percentage of species in each crop was elaborated and then was separated along the first two axes of the scatter plot of non-metric multidimensional analysis based on Gower similarity measure of species in the ten habitats.

3. RESULTS

3.1. General distribution

Appendix 1 shows the spatial distribution of the plant species recorded in the surveyed area. It is evident that the number of the species and their presence performance varied from site to site. In unsubmerged islands (sites), the total number of recorded species ranged from 68 to 79 species except for El-Foqaey island (S3) and Baget Saleh island (S15) where they recorded 54 and 46 species respectively. At the contrary, in the submerged islands, the total number of species ranged between 33 and 53 species except for Bani-Soliman island (S7) that included 68 species. The uncultivated submerged island, Al Shoqr island (S4), included only 36 species.

The species were grouped in generalized five categories (I-V) of presence performance. Category V, species recorded in 13-15 sites, had the widest ecological amplitude, included 13 species (c. 8.5 % of the total recorded species). Among these, six species, namely *Cynodon dactylon* (L.) Pers., *Chenopodium album* L., *Senecio aegyptius* L. var. *discoideus* Boiss., *Cyperus rotundus* L. var. *rotundus*, *Rumex dentatus* L. subsp. *dentatus* and *Vossia cuspidata* (Roxb.) Griff. were recorded in all sites with presence performances ranged between 98.7 and 23.4%. Of these, *Cynodon dactylon* had the widest ecological amplitude with the highest performance, P = 98.7 %. In addition, five species were recorded in 14 sites with presence values ranged between 26% and 37.7%. These were 2 winter weeds, *Malva parviflora* L. and *Sonchus oleraceus* L.; and 3 canal bank species, *Pluchea dioscoridis* (L.) DC., *Phragmites australis* (Cav.) Trin. ex Steud. subsp. *australis* and *Rorippa palustris* (L.) Besser. Two species, namely *Persicaria lapathifolia* (L.) Gray and *Solanum nigrum* L. var. *nigrum*, were recorded in 13 sites with presence values 29.9% and 39 % respectively.

Category IV, species recorded in 10-12 sites, included 18 species (c. 11.8%). The presence performance values ranged between 36.4% and 15.6%. Seven species were collected from 12 sites; six species were recorded in 11 sites and five species from 10 sites. Except for the 4 winter weeds, *Chenopodium murale* L., *Dactyloctenium aegyptium* (L.) Willd., *Capsella bursa-pastoris* (L.) Medik. and *Cichorium endivia* L. subsp. *divaricatum* Schousb. and the 3 summer weeds, *Echinochloa colona* (L.) Link, *Portulaca oleracea* L. and *Amaranthus viridis* L., the rest 11 species were canal bank species.

Category III, species recorded in 7-9 sites, included 20 species or 13.2 % of the total collected species. The presence performance values ranged between 33.8% and 11.7%. Seven species were recorded in 9 sites, four in 8 sites and 9 in 7 sites. Of these, two species showed high presence values in definite sites as *Ludwigia stolonifera* (Guill. & Perr.) P.H.Raven that recorded 100% in site 4 (Al Shoqr island) and *Polypogon monspeliensis* (L.) Desf. that recorded 80% in site 6 (Sannor island).

Category II, species recorded in 4-6 sites, included 39 species; ten species in 6 sites, 21 species in 5 sites and eight species in 4 sites with presence performances ranged between 20.8% and 5.2%.

Category I, species recorded in 1-3 sites, included 62 species (c. 40.8% of the total recorded species). These species represented the most narrow-spread species. Eleven species were collected from three sites, 16 species were recorded in two sites and 35 species were confined to only one site. Of these, 24 of species were recorded in the permanent islands especially in Al Koraymate island (S13) that included 10 species. Also the records showed that the presence values of these species ranged between 1.3 and 9.1%. Again, some species might show high performances in definite sites as the hydrophyte *Myriophyllum spicatum* L. that recorded 60% in site 9 (Beni-Suef island), the canal bank *Silybum marianum* (L.) Gaertn. var. *marianum* that recorded 57.1% in site 5 (Beba island) and *Orobanche crenata* Forssk. that recorded 50% in site 2 (Awlad Shaker submerged temporary island).

3.2. Crop-weed relationships (Sociological range)

The floristic composition differed from one habitat (orchard, farmland, canal bank and waste land) to another, also the presence performances of the species. Moreover, the distribution of species among different habitats showed high variability, the highest was 107 species in canal banks followed by the waste lands, 99 species, while the lowest was 27 species in onion farmlands. The total number of species ranged between 32 and 60 in orchards and between 27 and 60 in crop farmlands. Appendix 2 summarized the presence performance of each species within the studied ten habitats. Category 1 (widest sociological range of species) included one species; *Cynodon dactylon* that was recorded in all 10 habitats with high performance (P = 97.4%).

Category 2 included four species that existed in 9 habitats with presence performances ranged between 39% and 64.9%. But, the behavior of the species differed from habitat to another. For instance, the four species recorded their high performances (P = 100%) in mango orchards, *Chenopodium album* recorded high performances in wheat and onion farmlands while *Cyperus rotundus* var. *rotundus* and *Malva parviflora* recorded that in lupin farmlands and orange orchards respectively.

Category 3 included 11 species that recorded in 8 habitats with presence performance values ranged between 15.6% and 59.7%. The canal bank species, *Senecio aegyptius* var. *discoideus*, *Pseudognaphalium luteoalbum* (L.) Hilliard & B. L. Burttand and *Pluchea dioscoridis* performed better in waste lands and canal banks than in other habitats. While, the mesophytic species like *Amaranthus viridis*, *Chenopodium murale*, *Convolvulus arvensis* L. and *Oxalis corniculata* L. performed better in orchards than in other habitats.

Category 4, existed in 7 habitats, included 10 species with presence performance values ranged between 10.4% and 32.5%. Among these, five species performed better or might record P = 100% in orchards whereas monitored in other habitats with very low performances. These were *Echinochloa colona*, *Portulaca oleracea*, *Euphorbia helioscopia* L., *Euphorbia peplus* L. and *Bidens pilosa* L. On the other hand, *Melilotus indicus* (L.) All. performed better in stands of clover, onion and waste lands than in other habitats.

Category 5 (species present in six habitats) included 10 species with presence performance values ranged between 7.8% and 39%. *Echinochloa stagnina* (Retz.) P. Beauv., *Capsella bursa-pastoris* (L.) Medik., *Mentha longifolia* (L.) Huds. subsp. *typhoides* (Briq.) Harley, *Stellaria pallida* (Dumort.) Murb. and *Dactyloctenium aegyptium* recorded the highest presence (P = 100%) in mango orchards. On the other hand, *Eclipta prostrate* (L.) L., *Persicaria lapathifolia* (L.) Gray and *Cyperus alopecuroides* Rottb. showed certain consistency in the canal bank with high performance ranged between 53.3% and 93.3%.

Category 6 included 19 species with presence performances ranged between 7.8% and 26%. Only 3 species recorded the highest presence performance (P = 100%) in certain habitats. These were the annuals *Amaranthus hybridus* L. subsp. *hybridus*, *Paspalidium geminatum* (Forssk.) Stapf and *Digitaria ciliaris* (Retz.) Koeler in mango orchards. Whereas, *Phragmites australis* subsp. *australis* showed high performance in canal bank (P = 93.3%) and *Cichorium endivia* subsp. *divaricatum* recorded 71.4% in clover farmlands.

Category 7 included 13 species that presented in four habitats with presence performances ranged between 5.2% and 23.4%. *Phalaris paradoxa* L. was the only species that recorded performance of 100% in mango orchards. Also, *Glinus lotoides* L., *Persicaria lanigera* (R. Br.) Soják, *Leptochloa fusca* (L.) Kunth, *Potentilla supina* L. and *Homognaphalium pulvinatum* (Delile) Fayed & Zareh preferred existence in waste lands with P values ranged between 63.6% and 36.4%. *Euphorbia heterophylla* L. had its highest P values in orchards.

Category 8 included 15 species with presence performances ranged between 3.9% and 14.3%. *Urtica urens* L. was the only species that recorded P = 100% in mango and orange orchards. Also, *Corchorus olitorius* L. recorded showed high presence performance in banana orchards (P = 80%). *Avena fatua* L. recorded only in some winter crops, wheat and clover, and achieved P value 46.2% in wheat farmland.

Category 9 included 32 species with presence performances ranged between 2.6% and 23.4%. Among these, 13 species were confined to waste lands and canal banks. The most prominent species were *Vossia cuspidata*, *Cyperus articulatus*, *Ludwi-*

gia stolonifera and *Tamarix nilotica* (Ehrenb.) Bunge with performance values ranged between 27.3% and 100%.

Category 10 included 37 species that were confined to only one weed assemblage (narrowest sociological range) distributed as follows: 16 species in canal banks, 6 species in waste lands, 7 species in crop farmlands, 8 species in orchards.

3.3. Multivariate analyses

3.3.1. Ordination and clustering of the islands

The dendrogram resulting from TWINSPAN (Figure 2) of the 15 studied islands based on their floristic composition showed that two main groups were separated and the border line species was *Cyperus articulatus*. The first represented the unsubmerged (permanent) islands and was dominated by *Melilotus indicus*. This group was further differentiated into two subgroups (C and D), at the subsequent levels of classification. The second one comprised the submerged (temporary) islands; *Panicum coloratum* was the indicator species of this group. Also this group was differentiated into two subgroups (A and B) at the subsequent levels of classification.

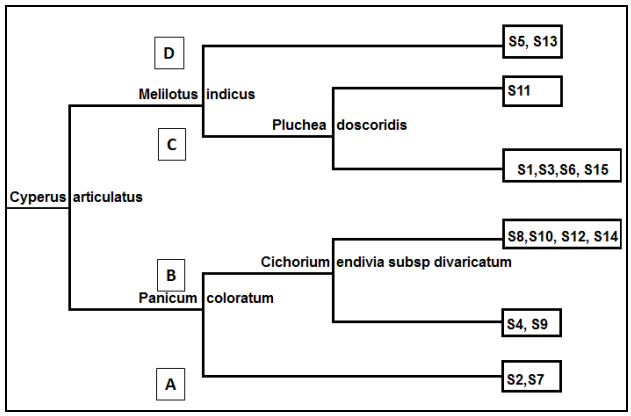

Fig. 2. TWINSPAN dendrogram of the 15 studied sites based on their species presence values. A-D are the 4 separated TWINSPAN floristic groups.

3.3.2. Similarity coefficient between the investigated islands

Analysis of the floristic composition of the investigated sites carried out by IBM SPSS correlated the distances between each two islands. The floristic correlation, similarities and dissimilarities, between the islets were expressed in Table 1. It was evident that even neighboring islands showed remarkable differences in their floristic composition, or had a small number of species in common.

Table 1. Similarity coefficient between the investigated islands (S1 – S15). For abbreviations, see Figure 1.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**
S1	100														
S2	39.7	100													
S3	60.4	43.9	100												
S4	36.9	49.2	31	100											
S5	47.1	14.7	43.7	29.4	100										
S6	45	21.8	54.5	39.4	48.8	100									
S7	43	73.9	44.7	52.6	25.8	31.1	100								
S8	42.9	61.6	46.1	51	23.1	36.9	68.3	100							
S9	52.5	58.4	47	48	24	41	58.3	59.7	100						
S10	44.1	43.1	46.3	44.9	41.3	55.6	50.3	66.6	52	100					
S11	51.6	25.8	64.2	31.5	50.7	52.5	28.5	27.1	32	38	100				
S12	43.6	62.1	47.2	45	30.5	31.1	62.6	60.3	44.9	50.4	28.8	100			
S13	49.9	15.5	59.7	25.5	60.3	53.3	17.9	32.2	19.2	43.7	55	26.1	100		
S14	45.6	54.6	53.2	48.3	31.6	35.4	63.3	70.7	52.3	64.2	27.6	71.8	35	100	
S15	58.3	40.6	56	43.1	57.8	60.6	46.9	48	47.6	70.9	49	45.7	63.5	57.3	100

Table 2. Mean values ± standard errors of the soil variables in the 15 selected islands representing the floristic groups A-D obtained by TWINSPAN. EC = electric conductivity, OM = organic matter. Significant (*P< 0.05), highly significant (**P< 0.01)

	TWINSPAN floristic groups					
	A	**B**	**C**	**D**	**F- ratio**	**P**
No. of sites	**2**	**5**	**6**	**2**		
Sand	91.0 ± 1.41	68.5 ± 14.05	64.4 ± 29.94	65.5 ± 2.12	0.874	0.004**
Silt	6.5 ± 0.71	19.17 ± 7.88	19.8 ± 14.82	20.5 ± 0.71	0.927	0.461
Clay	2.5 ± 0.71	12.33 ± 6.22	15.8 ± 15.07	13.5 ± 2.12	0.848	0.149
pH	8.15 ± 0.21	8.2 ± 0.27	8.16 ± 0.22	8.31 ± 0.43	0.172	0.917
EC	0.5 ± 0.71	1.00 ± 2.00	5.2 ± 6.3	6.5 ± 7.78	1.302	0.003**
OM	0.11 ± 0.08	2.42 ± 3.04	0.56 ± 0.64	3.1 ± 0.57	1.393	0.002**
HCO_3	1.71 ± 1.82	0.75 ± 0.67	1.44 ± 1.23	4.06 ± 4.02	2.182	0.064*
Cl	4.6 ± 3.68	6.02 ± 11.76	48.88 ± 58.24	52.49 ± 65.92	1.444	0.052*
SO_4	2.0 ± 0.00	3.17 ± 5.85	10.0 ± 12.06	20.5 ± 24.75	1.456	0.283
Ca	1.89 ±1.25	2.20 ± 3.12	9.5 ± 11.05	20.96 ± 25.66	1.873	0.091*
Mg	1.00 ± 0.00	1.00 ± 2.00	6.20 ± 7.98	11.50 ± 14.85	1.543	0.265
Na	4.07 ± 2.95	6.84 ± 12.75	43.91 ± 52.31	41.96 ± 51.97	1.314	0.046*
K	1.00 ± 1.41	0.33 ± 0.52	0.80 ± 0.84	2.50 ± 2.12	2.448	0.121
NO_3	0.43 ± 0.30	0.30 ± 0.33	0.09 ± 0.05	0.20 ± 0.02	1.159	0.073*
PO_4	0.00 ± 0.00	0.33 ± 0.52	0.00 ± 0.00	0.00 ± 0.00	1.1	0.392

The similarity values differed. In permanent islands the lowest similarity value was 45% recorded between Zawet Elgedamy island (S1) and Sannor island (S6) and the highest value was 64.2% recorded between El-Foqaey island (S3) and El-Alalma island (S11), while this might reach 73.9% as in between the submerged islands (S2; Awlad Shaker island and S7; Bani-Soliman island). On the contrary, these values were remarkably low, in between the submerged and unsubmerged islands that might reach 14.7% or 15.5% as in the correlation between S2 with either S5 or S13 respectively. Unexpectedly, the similarity coefficient between the unsubmerged island Baget Saleh (S15) showed relatively high consistency values even with the submerged islands ranged between 40.6% and 70.9%.

3.3.3. Species-soil relationships

Based on the resultant TWINSPAN 4 groups A-D, the investigated 15 soil parameters were checked to understand the effect of soil variables on the distribution of the species among the monitored islands. Table 2 indicates that the sand content of the soil, electric conductivity (EC) and organic matter (OM) were highly significant. In addition, 5 variables namely, HCO_3, NO_3, Cl, Ca and Na were significant. Moreover, it was evident that sites of groups A (*Panicum coloratum* was the leading dominant species) and C (*Pluchea dioscoridis* was the leading dominant species) were correlated with the high sand content of the soil that might record 92.41% and 95.34% respectively. While the sites of group B (*Cichorium endivia* subsp. *divaricatum* was the leading the dominant species) was affected by the organic matter of the soil. Group D (*Melilotus indicus* was the leading dominant species) was correlated with the bicarbonates, calcium and magnesium contents of the soil.

The relation between the flora and soil variables was indicated on the ordination diagram produced by Canonical Correspondence Analysis (CCA). The length and direction of an arrow representing a given environmental variable provide an indication of the importance and direction of the

gradient of environmental change for that variable, within the set of the samples measured. The angle between an arrow and each axis is a reflection of its degree of correlation with the axis.

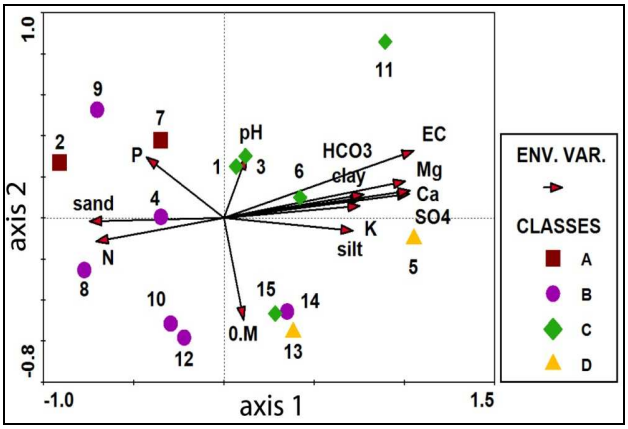

Fig. 3. CCA biplot of axis 1 and 2 showing the distribution of the 15 sites with their TWINSPAN floristic groups (A-D) in relation to the soil variables.

CCA ordination biplot with floristic groups (A-D) and the examined soil variables were shown in Figure 5. Preliminary analysis revealed high inflation factors for 3 soil variables (clay, chlorides and sodium) which should be excluded from the analysis. So, this analysis is based on 12 soil parameters: sand, silt, organic matter, pH, electric conductivity, sulfates, bicarbonates, phosphates, nitrates, calcium, magnesium and potassium contents.

It can be noticed that sites of group A were highly correlated with sand and phosphates; group B was correlated with sand, nitrogen, organic matter and phosphates; while group C was affected by pH, electric conductivity, magnesium, and bicarbonates but sites of group D were affected just by silt and organic Matter. These results revealed an association between floristic composition and the measured soil variables.

Table 3 showed the interset correlation of the soil variables along the 4 axes of the CCA biplot ordination. It is obvious that CCA axis 1 was highly positively correlated with the electric conductivity (0.6465) and highly negatively (-0.4574) correlated with sand content of the soil. This axis can be called electric conductivity - sand axis. Also, CCA axis 2 was highly positively (0.2557) correlated with the electric conductivity and highly negatively (-0.3891) correlated with organic matter. Thus, this axis can be interpreted as the electric conductivity - organic matter axis.

3.3.4. Ordination of habitats

By using the ordination of the Community Analysis Package (CAP), the ten different habitats-crop farmlands were clustered in 4 groups (Figure 4). Group A comprised the orchards, group B comprised the winter crops, group C comprised the summer crop and group D comprised the habitats of the canal banks and waste lands.

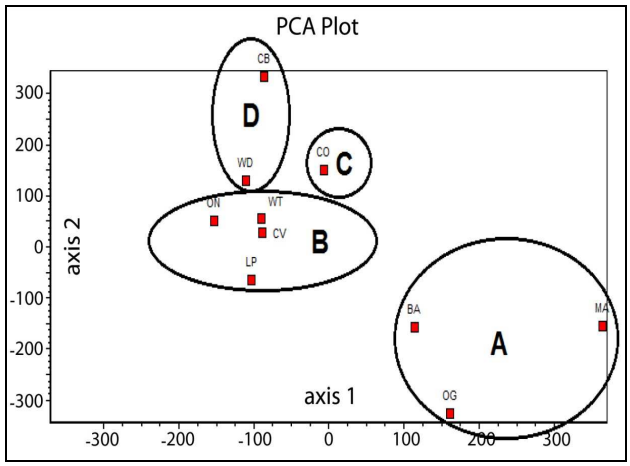

Fig. 4. PCA ordination of the investigated ten habitats. A-D are the groups that resulted from cluster analysis. For abbreviations, see Table 4.

3.3.5. Similarity coefficient of habitats

Table 4 showed the similarity coefficient between each pair of the investigated ten habitats-farmlands. The lowest values were between orange orchards and both waste lands and canal banks where they recorded 10% and 10.9% respectively. Also, the affinity coefficients recorded 11.1 % between Mango orchards and each of Onion farmlands and waste lands. On the other hand the highest values (74.4%) were between wheat and clover, both were winter crops. This was the case also in the correlation between banana and orange orchards (72.5%).

Table 3. The interset correlations of the soil variables along the 4 axes of the CCA biplot ordination, eigenvalues and species-environmental correlation coefficients.

	AX1	**AX2**	**AX3**	**AX4**
Eigenvalues	**0.2257**	**0.03**	**0.0914**	**0.1067**
species-environmental correlation coefficient	**0.9362**	**0.9968**	**0.9994**	**0.9939**
sand	-0.4574	-0.0127	0.4764	0.2452
silt	0.4389	-0.049	-0.48	-0.231
clay	0.472	0.0761	-0.4679	-0.2573
OM	0.0668	-0.3891	-0.1224	0.1499
pH	0.0763	0.235	-0.2483	0.4373
EC	0.6465	0.2557	-0.1728	0.0562
HCO_3	0.4763	0.0897	0.1768	0.4641
SO_4	0.6311	0.0919	-0.1133	0.2498
Ca	0.634	0.1031	-0.065	0.3424
Mg	0.6173	0.1395	-0.1234	0.2734
K	0.4624	0.0454	0.2763	0.456
NO_3	-0.4355	-0.0895	0.2848	-0.089
PO_4	-0.264	0.231	-0.43	0.5524

Table 4. The similarity coefficient between each pair of the investigated habitats by using the correlation of SPSS program. MA = Mango, BA = Banana, OG = Orange, WT = Wheat, CV = Clover, CO = Corn, ON = Onion, LP = Lupin, CB = Canal banks and WD = Waste lands.

	MA	**BA**	**OG**	**WT**	**CV**	**CO**	**ON**	**LP**	**CB**	**WD**
MA	100									
BA	63.5	100								
OG	52.6	72.5	100							
WT	30.5	43.6	30.7	100						
CV	31.3	46.8	31	74.4	100					
CO	48.9	58.1	50.4	20.4	29.4	100				
ON	11.1	20.6	3.9	62	63.4	16.5	100			
LP	30.2	39.4	29	50.8	66	43	61.4	100		
CB	17.5	26.1	10.9	36.9	38.1	34.5	40	45.1	100	
WD	11.1	25.9	10	46.9	54.3	29.9	53.7	55.7	67.1	100

4. DISCUSSION

In this study, altogether 152 species belonging to 117 genera in 49 families of the vascular plants were recorded. It was evident that the number of species and also the presence performance varied from site (island) to another. It was obvious that even neighboring islands showed remarkable differences in their floristic composition, or had small number of species in common. When the total number of species might reach 79 species in permanent (unsubmerged) islands, it might decrease to be 33 species in temporary islands and this was not surprising considering that the unsubmerged islands were subjected to land cultivation through the whole year while the submerged islands were only

cultivated in winter months when the Nile water level was convenient, from November to June in most years. Kim et al. [77] revealed that anthropogenic disturbance of natural sites, here was the extensive cultivation in unsubmerged islands than submerged ones, had a considerable effect on species richness. This is in accordance with the investigation on the flora of Berlin [78-80], central Europe [81] and Thienemann's principle that "the more variable the habitat condition, the higher the diversity in a biocoenosis" [82]. The ecological amplitude of species varied, some showed broad while others were confined to special type of islands with very narrow amplitude. Six species, namely *Cynodon dactylon*, *Chenopodium album*, *Senecio aegyptius* var. *discoideus*, *Cyperus rotundus* var. *rotundus*, *Rumex dentatus* subsp. *dentatus* and *Vossia cuspidata* were recorded in all islands with different presence performance values ranged between 23.4% and 98.7% and had the broadest ecological amplitude. This result was also conducted by Shaheen [83] who cited that these weeds were ubiquitous, or as mentioned by Shaltout and Sharaf El-Din [84] who revealed that the species with large amplitude as *Cynodon dactylon* was often caused by phenotypic plasticity and heterogeneity. In addition, Abd El-Ghani [46] cited that the abundance of Vossia cuspidata in the sites of his surveyed area (islands of the Nile valley, Egypt) might be attributed to its position at the opposite ends of environmental gradients as mentioned by Muller-Dumbois and Ellenberg [85]. On the other hand, weeds of moderate occurrence might be related to the need for special habitats "thermic preferability" [83]. The existence of some species in a few sites as Myriophyllum spicatum might be related to the fluctuations of the water level, cleaning practices and human activities as fishing and boating [86]. Also the restriction of some species to special one site of the studied area as *Najas marina* subsp. *armata* in site 9 might be related to the spread of extensive agricultural practices that represent an acute loss of habitats for these species and therefore may be replaced by *Myriophyllum spicatum*. This is the same result that was reported by Agami and Wise [87], or to the allelopathic effect of *Myriophyllum spicatum* [88, 89].

Using of twinspan technique gave a clear cut between the submerged and unsubmerged permanent islands. It divided the studied islands into 2 groups depending on the floristic composition. Our results indicated an important role of habitat diversity in shaping floral diversity patterns in most species subsets. The similarity coefficient between the floristic compositions of each pair of the islands indicated the high correlation between the submerged islands that might reach 73.9% and between the unsubmerged islands that might reach 64.2%, while it recorded 14.7% between the submerged and unsubmerged islands. This might be related to the length of cultivation time. Unsubmerged permanent islands are cultivated through the whole year in contrary to the habitat loss in the submerged islands during the un-cultivation time. The strong correlation of plant species richness with habitat diversity had been documented by many authors [90-92].

One of the most important gradients in weed species composition in this study is the type of crop. This is confirmed by many authors [93-95]. In Egypt, 2 crops are usually grown in a seasonal sequence: a winter crop and a summer crop. It results that a crop rotation is accompanied by a weed-flora rotation [12]. The agro-ecosystem of the studied area can be differentiated into orchards and croplands. As demonstrated by the ordination of the species of the different habitats, the crop type plays an important role in the structure of the weed community. The role of the crop type is indicated by the restriction of the parasitic weed species to specific crop, for instance *Orobanche* sp. with clover or because the weed requirements to a definite exudates secreted by the roots of its host as *Orobanche crenata* with *Vicia faba* [96]. Some species are more abundant in certain crops with which they exhibit similarity morphologically and phenologically (e.g., *Avena fatua* L. in wheat crop) such similarity makes the recognition of the weed species from the crop plants very difficult and consequently hinders its control [57]. The dominance of the weed species with discoid stems such as *Cichorium endivia* L. subsp. *divaricatum* Schousb. in clover could be related to the fact that this crop undergoes three to five cuts during its growth period. This is supported by Abd El-Ghani and El-Bakry [51]. Moreover, the protection given by the tree foliage of the orchards affects the environ-

ment of weeds. The orchards exhibit 2 different microhabitats according to light conditions: the shaded areas below the crowns of trees support the growth of shade-loving species such as *Poa annua*, *Stellaria pallida* and *Urtica urens* whereas the sunny microhabitats support the growth of other species belonging to croplands. Moreover, canal bank species such as *Mentha longifolia* subsp. *typhoides*, *Phragmites australis* subsp. *australis* and *Imperata cylindrical* can grow in the moist microhabitats produced by the shade of the crowns of trees. These findings were also confirmed [56, 97, 98] that such environmental microheterogeneity promotes the diversity.

The weed species vary in their sociological range, ecological aggressiveness and seasonal preference. Sociological range and ecological performance seem to be linked; most species in the first category (present in all assemblages) are also the species with higher performance values. Species with narrow sociological range present in a few assemblages often have low scores of performance values [56]. Species richness varied from one crop to another. The winter weeds represent the main bulk of the recorded species within each crop, this may be attributed to differences in the weed control methods of the two winter and summer crops, this is in agreement with Hegazy et al. [57]. On comparing orchards with field crops, it can be recorded that orchards have a relatively large number of perennials weeds and rhizomatous species than that of field crops, this can be attributed to different cultivation practices that orchards are rarely ploughed. The same result was achieved by Abd El-Ghani et al. [56] in their studies on olive orchards. Also, in the plantation of sugarcane in Ethiopia, attributed that to the wider spacing between trees rows, a long growth cycle and constant moist conditions due to irrigation, which might have created conductive conditions for the growth of weeds [99].

Most species that are dominant in the habitats of canal banks and waste lands are perennials as *Vossia cuspidata* (Roxb.) Griff., *Cyperus articulatus* L., *Ludwigia stolonifera* (Guill. & Perr.) P.H.Raven, *Tamarix nilotica* (Ehrenb.) Bunge, *Salix mucronata* Thunb., *Phoenix dactylifera* L. and *Juncus bufonius* L. This is supported by Shaltout [100] who mentioned that the dominance of these species in the abandoned areas may be attributed to their abilities to cope with the significant substrate alterations, which may inhibit the reestablishment of other long-lived species. These all support the view that increasing habitat heterogeneity increases species diversity [101]. The species that are confined to canal banks or water loving species or even hydrophytes as *Typha domingensis* (Pers.) Poir. ex Steud., *Azolla caroliniana* Willd., *Ceratophyllum demersum* L., *Plantago major* L. and *Myriophyllum spicatum* L. or restricted to salinized waste land habitats such as *Cyperus difformis* L., *Fimbristylis bisumbellata* (Forssk.) Bubaniand and *Polycarpon prostratum* (Forssk.) Asch. & Schweinf. can be attributed to habitat preference phenomenon. Abd El-Ghani and Fawzy [102] postulated this phenomenon in the farmlands of the Egyptian Oases.

The ordination carried out by MVSP followed by CAP classified the 10 habitats-farmlands into distinct 4 groups: the first included the orchards (orange, banana and mango), the second included the winter crop farmlands (wheat, clover, lupin and onion), the third group included the canal banks and waste land habitats and the corn cultivations, a summer crop, stood alone in the fourth group. In addition, highly significant correlations were recorded between individuals of each group. This result is in line with what reported in the studies of Abd El-Ghani et al. [56] in the reclaimed lands along the northern sector of the Nile valley in Egypt. Also, this assures the differentiation of the Egyptian weeds into 3 main categories according to their seasonal performance: winter, summer and all-year-weeds postulated by several authors [50, 51]. Moreover, it showed that the differences in weed species were mainly affected by type of crop, seasonal preference, and ecological factors.

The edaphic characteristics of the soil were among the most delimiting factors in the distribution of the flora in any particular area. It was found that the sand contents of the soil, electric conductivity, and organic matter were highly significant ($p < 0.01$) in addition to bicarbonates, chlorides, calcium, sodium and nitrogenous contents of the soil were also significant ($p < 0.05$) among the prevalent floristic groups of the area of study. The resultant Twinspan 4 floristic groups were affected by the soil variables. Groups A (*Panicum coloratum* was the leading dominant species) and C (*Pluchea dioscoridis* was the leading dominant species) were

correlated with sand content of the soil, group B (*Cichorium endivia* subsp. *divaricatum* was the leading dominant species) was correlated with the organic matter while group D (*Melilotus indicus* was the leading dominant species) was correlated with the bicarbonates, calcium and magnesium contents of the soil. Even within same soil condition, same floristic group, some species thrive well [49], in addition the probability of finding these species growing together might be great, even though other factors also influence their abundance, as climate, ability for competition, seed production, capacity and geographic distribution.

The application of Canonical Correspondence Analysis (CCA) on the matrix of species against the different habitats of the present study demonstrated the effect of soil variables on the spatial distribution of weed communities in the study area. It was obvious that the first axis of CCA biplot was positively correlated with the electric conductivity and negatively correlated with sand content of the soil. This axis can be interpreted as the electric conductivity - sand axis. Also, the second axis was positively affected by electric conductivity and negatively correlated with the organic matter thus can be interpreted as the electric conductivity - organic matter axis. The application of ordination and clustering by using Twinspan and Canonical Correspondence Analysis (CCA) summarized the large complex data and compared that with the environmental information [103]. This resulted in the division of the monitored islands into 2 clear sets. One included the submerged and the other included the unsubmerged islands which were subdivided into 2 subgroups. Also, it correlated these subgroups to the soil variables prevailing in the area of study [75, 104].

AUTHORS' CONTRIBUTION

All authors contributed in all stages of this work except for writing the manuscript which had been written by AS. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Appendix 1. The general distribution of the recorded species in the surveyed 15 sites. Sites are shown in Figure 1. Values are the average frequency percentages (f%) of each species in the site

Sites	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	SUM	
Species / Number of stands	**7**	**4**	**6**	**2**	**7**	**5**	**6**	**5**	**5**	**5**	**6**	**4**	**6**	**3**	**6**	**77**	**%**
Species that recorded in 13-15 sites																	
Cynodon dactylon	100	100	100	100	100	100	100	100	80	100	100	100	100	100	100	76	98.7
Chenopodium album	29	100	67	50	14	60	67	100	80	100	50	50	50	100	100	50	64.9
Senecio aegyptius var. discoideus	57.1	100	66.7	100	57.1	20	83.3	80	40	40	50.	100	33.3	100	33.3	46	59.7
Cyperus rotundus var. rotundus	71.4	25	16.7	50	71.4	40	50	60	80	60	16.7	25	50	66.7	66.7	39	50.6
Rumex dentatus subsp. dentatus	28.6	25	50	50	42.9	60	33.3	40	40	40	33.3	50	66.7	33.3	66.7	34	44.2
Vossia cuspidata	14.3	50	16.7	50	14.3	20	33.3	40	20	20	16.7	25	16.7	33.3	16.7	18	23.4
Malva parviflora	57.1	50	50	100	14.3	20	33.3	60	40	40	50	50	16.7	33.3		29	37.7
Sonchus oleraceus	42.9	50	33.3	50	14.3		33.3	20	40	40	33.3	25	16.7	33.3	33.3	23	29.9
Pluchea dioscoridis	42.9	75	16.7	50	14.3	20	33.3	60	40	20		25	16.7	33.3	16.7	22	28.6
Phragmites australis subsp. australis	28.6	50	16.7	50	28.6	20	33.3	20	20	20	33.3	25	16.7		33.3	20	26
Rorippa palustris	14.3	75	16.7	50	14.3	20	33.3	20	40	40	16.7	50	16.7	33.3		20	26
Persicaria lapathifolia	14.3	75	50		14.3	20	33.3	60	60	40		100	16.7	100	50	30	39
Solanum nigrum var. nigrum	28.6	75	33.3	100	14.3	20	33.3		40	20	33.3	25	33.3		33.3	23	29.9
Species that recorded in 10-12 sites																	
Echinochloa colona	14.3		16.7	50	85.7	40		20	20	40	50	25	50		50	25	32.5
Portulaca oleracea	28.6		16.7	50	57.1	40			20	40	33.3	25	50	33.3	33.3	22	28.6
Glinus lotoides	28.6	50	16.7	50	.	20	33.3	40	40	40		50		33.3	16.7	19	24.7
Eclipta prostrata	14.3	50	.	100	14.3	20	16.7	40	40	60		25	16.7		16.7	18	23.4
Persicaria senegalensis		25	16.7		14.3	20	33.3	20	40	20	16.7	25	16.7	33.3		14	18.2
Bidens pilosa	14.3	25		50	14.3		16.7	20	20	20	16.7	25	16.7	33.3		12	15.6
Dactyloctenium aegyptium	14.3			50	14.3	20	16.7	20	20	20	16.7	25	.	33.3	16.7	12	15.6
Pseudognphalium luteoalbum	71.4		50		57.1			20	20	40	50	25	66.7	33.3	50	28	36.4
Amaranthus viridis	42.9	75	16.7		.	20	50	40	80		50	75	16.7	33.3		25	32.5
Cyperus alopecuroides	14.3	50		50	57.1	40	33.3			20	33.3	50	50		33.3	22	28.6
Chenopodium murale	28.6		16.7		42.9	40			60	40	16.7	25	16.7	33.3	33.3	19	24.7
Persicaria salicifolia	14.3			100	14.3	40	16.7	40	40	40			16.7	66.7	16.7	17	22.1
Oxalis corniculata	28.6	25		50	14.3	20	33.3	40	40	20	16.7		16.7			15	19.5
Trifolium resupinatum var. resupinatum	42.9	50	33.3		42.9	20			60	20	50	25			16.7	20	26
Persicaria lanigera	28.6	50	16.7	100	14.3	20	33.3	20				75		66.7		17	22.1
Echinochloa stagnina	28.6		.	100	.	60	16.7	40	40	20			16.7	33.3	33.3	17	22.1
Capsella bursa-pastoris	14.3		16.7	100	28.6	20	33.3	20	60	20					16.7	15	19.5
Cichorium endivia subsp. divaricatum					28.6	40	16.7	20		80	16.7	25	16.7	33.3	16.7	15	19.5
Species that recorded in 7-9 sites																	
Polypogon monspeliensis	28.6		16.7	50	57.1	80				60	66.7		50		66.7	26	33.8
Melilotus indicus	42.9	50	33.3		.	60	33.3		60	40	33.3				16.7	20	26
Poa annua	14.3		16.7		57.1	60	16.7			40	16.7		33.3		50	18	23.4
Polypogon viridis	28.6	25	16.7	50	14.3	20					16.7		33.3		33.3	12	15.6
Mentha longifolia subsp. typhoides		50	16.7	50			16.7	20				75	16.7	33.3	16.7	11	14.3
Ludwigia stolonifera	14.3	25		100	.	20		20		20	16.7	25		33.3		10	13
Corchorus olitorius		25		50	14.3			20	20	20	16.7	25			16.7	9	11.7
Convolvulus arvensis	57.1		33.3		42.9	20		20			50		66.7		16.7	19	24.7
Conyza bonariensis			33.3		42.9	20		20		20	33.3		50	33.3		14	18.2
Potentilla supina		50		50	28.6		33.3	20	20	20			16.7			11	14.3
Paspalidium geminatum	14.3	50		50		20	33.3		20			50			16.7	11	14.3

Amaranthus hybridus subsp. hybridus	57.1								40	16.7	25	50	33.3	66.7	16	20.8
Cyperus articulatus	42.9		16.7		42.9	40				33.3		66.7		16.7	16	20.8
Euphorbia peplus	28.6		33.3			40			20	33.3		33.3		16.7	12	15.6
Ceratophyllum demersum	42.9		33.3		.	20	16.7		40	33.3	25				12	15.6
Lamium amplexicaule					28.6		16.7	40		60		25	16.7	33.3	11	14.3
Vicia sativa subsp. Sativa	14.3		16.7		28.6	20				16.7		66.7		16.7	11	14.3
Chenopodium ambrosioides	28.6			50	14.3	40			40		16.7		33.3			11	14.3
Stellaria pallida			16.7	50	.	40		20	20		33.3		33.3			10	13
Digitaria ciliaris	14.3					20	16.7	40		20		50	16.7			9	11.7
Species that recorded in 4-6 sites																
Euphorbia helioscopia	28.6		66.7		42.9	40					33.3		50			16	20.8
Amaranthus biltum subsp. emerginatus					57.1	60					16.7	25	33.3		16.7	12	15.6
Imperata cylindrica	28.6		16.7		42.9	20					16.7		33.3			10	13
Leptochloa fusca	14.3	50					16.7	40	60	20						10	13
Panicum coloratum	28.6	25			28.6		33.3				16.7			16.7		9	11.7
Ranunculus sceleratus	42.9		16.7		14.3						16.7		16.7		16.7	8	10.4
Ricinus communis	28.6	25				20		20		40				16.7		8	10.4
Brachiaria reptans	28.6	25					16.7	40				25	16.7		.	8	10.4
Datura stramonium		25		50	28.6		33.3		20						16.7	8	10.4
Homognaphalium pulvinatum							16.7	40	20	40		25	16.7			8	10.4
Eichhornia crassipes	42.9				42.9		16.7					75		33.3		11	14.3
Polygonum equisetiforme	28.6	100					33.3	40	20							11	14.3
Tamarix nilotica	28.6		33.3			20					33.3		33.3			9	11.7
Amaranthus graecizans				50	28.6		16.7		20		66.7					9	11.7
Potamogeton nodosus	14.3							40		40			33.3	33.3		8	10.4
Paspalum distichum						20	16.7	40		40		50				8	10.4
Riccia	14.3		16.7		14.3	20					50					7	9.1
Salix mucronata	14.3		16.7		.	60	16.7				16.7					7	9.1
Coronopus didymus			16.7		.	40		20		20	33.3					7	9.1
Emex spinosa	14.3				42.9	20					16.7		16.7			7	9.1
Euphorbia prostrata	14.3		33.3		14.3	20			40							7	9.1
Phalaris minor		25			28.6			20			16.7		33.3			7	9.1
Phalaris paradoxa	14.3			50	14.3						50		16.7			7	9.1
Phyla nodiflora	14.3		16.7		28.6	20							16.7			6	7.8
Euphorbia heterophylla	14.3		16.7		28.6						16.7		16.7			6	7.8
Cynanchum acutum subsp. acutum		25					16.7			20		25		33.3		5	6.5
Desmostachya bipinnata			16.7		14.3	20				20			16.7			5	6.5
Phoenix dactylifera	.	25					16.7	20	20		16.7					5	6.5
Polycarpon tetraphyllum	14.3			50			16.7	20	20							5	6.5
Populus euphratica	14.3		16.7		14.3	20					16.7					5	6.5
Sesbania sesban	14.3		16.7			20			20				16.7			5	6.5
Trigonella hamosa			50			20					50		50			10	13
Avena fatua	28.6				57.1		16.7								16.7	8	10.4
Xanthium strumarium	14.3					20							33.3		16.7	5	6.5
Avena barbata subsp. barbata					14.3			20	40		16.7					5	6.5
Potamogeton perfoliatus					14.3	40					16.7	25				5	6.5
Sorghum virgatum			.		28.6						16.7		16.7		16.7	5	6.5
Anagallis arvensis subsp. arvensis var. caerulea	14.3		16.7				16.7						16.7			4	5.2
Galinsoga parviflora	14.3		16.7		14.3	20										4	5.2

Species that recorded in 1-3 sites															
Myriophyllum spicatum	42.9				20			60						7	9.1
Silybum marianum var. marianum	14.3			57.1							16.7			6	7.8
Cyperus michelianus subsp. pygmaeus					20	16.7	60							5	6.5
Anagallis arvensis subsp. arvensis var. arvensis	28.6				20				20					4	5.2
Adiantum capillus-veneris					20				20	16.7				3	3.9
Azolla caroliniana		25				16.7						33.3		3	3.9
Cyperus difformis				14.3		16.7				16.7				3	3.9
Fimbristylis bisumbellata		25				16.7			20					3	3.9
Juncus bufonius	14.3								20		25			3	3.9
Juncus hybridus					20		20			16.7				3	3.9
Setaria verticillata				14.3							16.7		16.7	3	3.9
Typha domingensis	28.6		.							33				4	5.2
Alternanthera sessilis			16.7								33.3			3	3.9
Orobanche cernua						33.3				16.7				3	3.9
Orobanche crenata		50						20						3	3.9
Veronica anagalloides subsp. taeckholmionum	28.6							20						3	3.9
Vicia sativa subsp. nigra		25				16.7								2	2.6
Alhagi graecorum						16.7				16.7				2	2.6
Enarthrocarpus lyratus						16.7						33.3		2	2.6
Fumaria parviflora	14.3												16.7	2	2.6
Gnaphalium polycaulon						16.7					16.7			2	2.6
Lolium rigidum						16.7				16.7				2	2.6
Medicago sativa subsp. sativa				14.3		16.7								2	2.6
Oldenlandia capensis var. capensis		25				16.7								2	2.6
Oxystelma esculentum	.	25				16.7								2	2.6
Polycarpon prostratum	14.3					16.7								2	2.6
Tagetes minuta	14.3					16.7								2	2.6
Cenchrus echinatus											33.3			2	2.6
Amaranthus hybridus subsp. cruentus											16.7			1	1.3
Ammi majus											16.7			1	1.3
Bromus catharticus											16.7			1	1.3
Eleusine indica											16.7			1	1.3
Nothoscordum gracile											16.7			1	1.3
Plantago major											16.7			1	1.3
Senna alexandrina											16.7			1	1.3
Trianthema portulacastrum											16.7			1	1.3
Verbena supina											16.7			1	1.3
Spergularia marina												25		1	1.3
Sphaeranthus suaveolens var. abyssinicus												25		1	1.3
Urtica urens										50				3	3.9
Citrullus colocynthis										16.7				1	1.3
Leersia hexandra										16.7				1	1.3
Lemna gibba										16.7				1	1.3
Spirodela polyrhiza									20					1	1.3
Brassica nigra								20						1	1.3
Najas marina subsp. armata								20						1	1.3
Vicia narbonensis var. narbonensis								20						1	1.3

Veronica anagallis-aquatica							33.3									2	2.6
Brassica juncea							16.7									1	1.3
Lotus arabicus							16.7									1	1.3
Potamogeton pectinatus							16.7									1	1.3
Potamogeton crispus						20										1	1.3
Eragrostis cilianensis					28.6											2	2.6
Vicia monantha					28.6											2	2.6
Withania somnifera					14.3											1	1.3
Leptochloa panicea					14.3											1	1.3
Sinapis allionii					14.3											1	1.3
Eruca sativa			16.7													1	1.3
Nymphaea caerulea			16.7													1	1.3
Orobanche ramosa var. schweinfurthii		50														2	2.6
Medicago polymorpha var. vulgaris	14.3															1	1.3
Acacia nilotica subsp. nilotica	14.3										.					1	1.3
	79	45	54	37	70	68	68	49	51	53	69	45	74	33	46		

Appendix 2. Sociological range of the recorded species in the 10 different habitats. P% = presence performance. For abbreviations, see Table 4.

	Orchards			Crop farmlands					CB	WD	Sum	
Type of habitats	**MA**	**BA**	**OG**	**WT**	**CV**	**CO**	**ON**	**LP**				
Species / Number of stands	**2**	**5**	**2**	**13**	**14**	**7**	**4**	**4**	**15**	**11**	**77**	**%**
Species present in all 10 habitats												
Cynodon dactylon	100	100	100	100	100	100	50	100	100	100	75	97.4
Species present in 9 habitats												
Chenopodium album	100	40		100	71.4	42.9	100	25	53.3	63.6	50	64.9
Cyperus rotundus var. *rotundus*	100	60	50		42.9	85.7	50	100	60	63.6	40	51.9
Rumex dentatus subsp. *dentatus*	100	60	50	53.8	78.6		50	50	26.7	36.4	36	46.8
Malva parviflora	100	60	100	46.2	57.1	28.6		50	20	18.2	30	39
Species present in 8 habitats												
Senecio aegyptius var. *discoideus*		40	50	69.2	50		100	50	73.3	90.9	46	59.7
Pseudognphalium luteoalbum	50	40		30.8	21.4		25	25	53.3	81.8	29	37.7
Polypogon monspeliensis	100	20		38.5	50		75	75	26.7	27.3	28	36.4
Amaranthus viridis	100	100	100	7.7	28.6	85.7			26.7	9.1	25	32.5
Pluchea dioscoridis		20		15.4	7.1	14.3	25	25	73.3	45.5	23	29.9
Solanum nigrum var. *nigrum*	100	40			35.7	14.3	25	25	40	45.5	23	29.9
Trifolium resupinatum var. *resupinatum*		40	50	38.5	35.7		25	25	13.3	27.3	20	26
Chenopodium murale	100	60	100	23.1	35.7			25	6.7	18.2	19	24.7
Convolvulus arvensis	100	60	100	15.4	7.1	42.9			26.7	18.2	19	24.7
Oxalis corniculata	100	80	50	7.7	7.1	28.6			26.7	9.1	16	20.8
Vicia sativa subsp. *sativa*		40	50	23.1	14.3		25	25	6.7	9.1	12	15.6
Species present in 7 habitats												
Echinochloa colona	100	60	100			100		25	46.7	27.3	25	32.5
Sonchus oleraceus	100	80		38.5	35.7	28.6			20	27.3	24	31.2
Portulaca oleracea	100	60	100	.	.	100		25	20	36.4	22	28.6
Melilotus indicus		40		23.1	42.9		50	25	13.3	45.5	21	27.3
Poa annua	100	20	100	38.5	28.6				13.3	18.2	18	23.4

Euphorbia helioscopia	50	80	100	15.4	21.4				13.3	18.2	16	20.8
Euphorbia peplus	100	60	50	15.4	14.3				6.7	18.2	13	16.9
Bidens pilosa	100	40	50	7.7	14.3	14.3			20		12	15.6
Polygonum equisetiforme		20	50	15.4	7.1			25	20	18.2	11	14.3
Datura stramonium	50	20	50	7.7		14.3			13.3	9.1	8	10.4
Species present in 6 habitats												
Persicaria lapathifolia				53.8	50		50	25	53.3	45.5	30	39
Cyperus alopecuroides					7.1	14.3	25	25	93.3	36.4	22	28.6
Eclipta prostrata	50				7.1	.	25	25	60	45.5	18	23.4
Echinochloa stagnina	100	20			7.1	14.3			53.3	36.4	17	22.1
Capsella bursa-pastoris	100	40	50	46.2	21.4					9.1	15	19.5
Conyza bonariensis	50	40		23.1				25	26.7	9.1	12	15.6
Mentha longifolia subsp. *typhoides*	100	40	50	15.4	7.1				20		11	14.3
Stellaria pallida	100	60	100	7.7	.				13.3	9.1	11	14.3
Dactyloctenium aegyptium	100	40			.	28.6	25		6.7	18.2	10	13
Euphorbia prostrata	50	20	50		7.1	14.3			6.7		6	7.8
Species present in 5 habitats												
Phragmites australis subsp. *australis*	50	20		15.4					93.3	18.2	20	26
Rorippa palustris		20		7.7	50				33.3	54.5	20	26
Persicaria salicifolia		20		38.5	7.1				46.7	27.3	17	22.1
Amaranthus hybridus subsp. *hybridus*	100	20			14.3	57.1			46.7		16	20.8
Cichorium endivia subsp. *divaricatum*				7.7	71.4	.	25	25	.	9.1	14	18.2
Persicaria senegalensis				15.4	7.1	.	25		53.3	18.2	14	18.2
Paspalidium geminatum	100			7.7		28.6			13.3	45.5	12	15.6
Chenopodium ambrosioides	50	60			7.1				26.7	18.2	11	14.3
Lamium amplexicaule	50	20		23.1	28.6				13.3		11	14.3
Trigonella hamosa				15.4	28.6		50		6.7	18.2	11	14.3
Imperata cylindrica	50	40		7.7					26.7	9.1	9	11.7
Panicum coloratum		20	50			28.6			13.3	27.3	9	11.7
Digitaria ciliaris	100	40				28.6		25	6.7		8	10.4
Paspalum distichum				7.7	7.1	14.3			13.3	27.3	8	10.4
Brachiaria reptans		40	50			28.6			6.7	9.1	7	9.1
Coronopus didymus		20	50	7.7	21.4				6.7		7	9.1
Emex spinosa	50	40	50	7.7					13.3		7	9.1
Phalaris minor		20		15.4	7.1			25	.	18.2	7	9.1
Xanthium strumarium	50	20	50	7.7					13.3		6	7.8
Species present in 4 habitats												
Glinus lotoides					21.4			50	40	63.6	18	23.4
Persicaria lanigera		20			21.4				46.7	54.5	17	22.1
Polypogon viridis				38.5	28.6				26.7	18.2	15	19.5
Leptochloa fusca							25	25	33.3	36.4	11	14.3
Potentilla supina	50				14.3				20	45.5	11	14.3
Amaranthus graecizans					14.3	14.3			20	18.2	8	10.4
Homognaphalium pulvinatum				15.4			25		6.7	36.4	8	10.4
Phalaris paradoxa	100	20		23.1					13.3		8	10.4
Phyla nodiflora	50					14.3			13.3	27.3	7	9.1
Euphorbia heterophylla	50	60	50		7.1						6	7.8
Avena barbata subsp. *barbata*		20		15.4			25			9.1	5	6.5
Silybum marianum var. *marianum*	50			7.7					6.7	18.2	5	6.5
Cynanchum acutum subsp. *acutum*					7.1	14.3			6.7	9.1	4	5.2

Species present in 3 habitats											
Amaranthus biltum subsp. *emerginatus*					21.4				26.7	36.4	11	14.3
Ranunculus sceleratus					14.3				33.3	18.2	9	11.7
Ricinus communis					7.1				33.3	27.3	9	11.7
Avena fatua				46.2	7.1					9.1	8	10.4
Corchorus olitorius		80	50			28.6					7	9.1
Desmostachya bipinnata							25		13.3	18.2	5	6.5
Sorghum virgatum				15.4					6.7	18.2	5	6.5
Urtica urens	100	20	100								5	6.5
Anagallis arvensis subsp. *arvensis* var. *caerulea*	50	20			14.3						4	5.2
Galinsoga parviflora	50								13.3	9.1	4	5.2
Vicia sativa subsp. *nigra*				15.4	7.1				.	9.1	4	5.2
Adiantum capillus-veneris	50	20			.				6.7		3	3.9
Orobanche cernua					7.1		25			9.1	3	3.9
Populus euphratica		20							6.7	9.1	3	3.9
Veronica anagalloides subsp. *taeckholmiorum*				7.7					6.7	9.1	3	3.9
Species present in 2 habitats												
Vossia cuspidata									100	27.3	18	23.4
Cyperus articulatus									60	54.5	15	19.5
Ludwigia stolonifera									46.7	27.3	10	13
Tamarix nilotica									33.3	45.5	10	13
Riccia aegyptica									13.3	54.5	8	10.4
Salix mucronata									26.7	36.4	8	10.4
Cyperus michelianus subsp. *pygmaeus*									6.7	36.4	5	6.5
Phoenix dactylifera									20	9.1	4	5.2
Alternanthera sessilis									6.7	18.2	3	3.9
Juncus bufonius									13.3	9.1	3	3.9
Alhagi graecorum									6.7	9.1	2	2.6
Enarthrocarpus lyratus									6.7	9.1	2	2.6
Tagetes minuta									6.7	9.1	2	2.6
Polycarpon tetraphyllum		20								36.4	5	6.5
Sesbania sesban						14.3			26.7		5	6.5
Anagallis arvensis subsp. *arvensis* var. *arvensis*					21.4					9.1	4	5.2
Juncus hybridus							25		13.3		3	3.9
Orobanche crenata					14.3					9.1	3	3.9
Setaria verticillata	100							25			3	3.9
Brassica nigra	50									9.1	2	2.6
Cenchrus echinatus	50								6.7		2	2.6
Eragrostis cilianensis						14.3			6.7		2	2.6
Eruca sativa	50			7.7							2	2.6
Fumaria parviflora		20			7.1						2	2.6
Gnaphalium polycaulon	50									9.1	2	2.6
Medicago polymorpha var. *vulgaris*				7.7						9.1	2	2.6
Oldenlandia capensis var. *capensis*					7.1					9.1	2	2.6
Orobanche ramosa var. *schweinfurthii*					7.1					9.1	2	2.6
Sinapis allionii				7.7					6.7		2	2.6
Veronica anagallis-aquatica		20								9.1	2	2.6
Vicia monantha				7.7						9.1	2	2.6
Withania somnifera				7.7					6.7		2	2.6

Species present in canal bank												
Typha domingensis									33.3		5	6.5
Acacia nilotica subsp. *nilotica*									6.7		1	1.3
Azolla caroliniana									6.7		1	1.3
Ceratophyllum demersum									6.7		1	1.3
Eichhornia crassipes									6.7		1	1.3
Lemna gibba									6.7		1	1.3
Myriophyllum spicatum									6.7		1	1.3
Najas marina subsp. *armata*									6.7		1	1.3
Plantago major									6.7		1	1.3
Senna alexandrina									6.7		1	1.3
Potamogeton crispus									6.7		1	1.3
Potamogeton nodosus									6.7		1	1.3
Potamogeton pectinatus									6.7		1	1.3
Potamogeton perfoliatus									6.7		1	1.3
Spergularia marina									6.7		1	1.3
Spirodela polyrhiza									6.7		1	1.3
Species present in waste lands												
Cyperus difformis										27.3	3	3.9
Fimbristylis bisumbellata										18.2	2	2.6
Lolium rigidum										18.2	2	2.6
Polycarpon prostratum										18.2	2	2.6
Lotus arabicus										9.1	1	1.3
Sphaeranthus suaveolens var. *abyssinicus*										9.1	1	1.3
Species present in crop farmlands												
Brassica juncea				7.7							1	1.3
Medicago sativa subsp. sativa					7.1						1	1.3
Citrullus colocynthis						14.3					1	1.3
Nymphaea caerulea							25				1	1.3
Vicia narbonensis var. *narbonensis*							25				1	1.3
Leptochloa panicea								25			1	1.3
Oxystelma esculentum								25			1	1.3
Species present in orchards												
Ammi majus	50										1	1.3
Bromus catharticus	50										1	1.3
Eleusine indica	50										1	1.3
Nothoscordum gracile											1	1.3
Trianthema portulacastrum	50										1	1.3
Verbena supina	50										1	1.3
Amaranthus hybridus subsp. *cruentus*	50										1	1.3
Leersia hexandra			50								1	1.3
Total number of species	58	60	32	56	60	31	27	29	107	99		