2. Review of Literature
Extreme environments are characterized by physicochemical variables that are deleterious to the otherwise non-adapted life forms. High salinity, extremes of pH, temperature and pressure, lethal radiation and absence of oxygen and water are some attributes associated with such biomes. Though considered initially as devoid of any life forms, a wide diversity of microorganisms, termed as ‘extremophiles’ were later found to inhabit these regions. Extremophilic communities are populated mostly by unicellular microorganisms, typically bacteria and Archae. The diversity of multicellular organisms in such communities is low.

The soil is categorized as ‘saline’ when the electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeds 4 dS m⁻¹ (approximately 40 mM NaCl) at 25 °C and has exchangeable sodium of 15%. Hypersaline regions contain salt concentrations over and above that of seawater i.e. 3.5% TDS. Resulting primarily from evaporation of sea water, hypersaline bodies are mostly thalassic, with salt concentrations proportional to that of the sea. In athallasic bodies, which are less abundant, the salt concentration is lower than that of sea water. The Great Salt Lake (USA) and the Dead Sea (Middle East) are two of the most researched hypersaline lakes. Hypersaline environments also include evaporation ponds found along the Red Sea coast, solar salterns and alkaline soda brines such as the Great Basin lakes of the western United States (Javor 1989), subterranean evaporite deposits and deep-sea basins found in the Red Sea. Intense UV radiation, desiccation, poor soil nutrient content and extremes of temperature are some of the features defining hypersaline habitats. Owing to their aridity, low nutrient content and absence/ negligible amount of organic matter, hypersaline soils possess very low vegetation cover consisting mostly of wild shrubs and grasses. At high salinities the yield of most crop plants is low, although many crops exhibit yield reduction at lower ECe (Munns 2005; Jamil et al. 2011). Globally, high salinity affects around 20% of total cultivated and 33% of irrigated agricultural lands, with an annual increase rate of about 10% on account of longer duration of warm climate leading to high surface evaporation, irregular rainfall patterns, shortage of clean water for irrigation thereby increasing the dependence on saline water, and unsustainable agricultural cultural practices. As per rough estimates,
at least 50% of the arable land is expected to be salinized by the year 2050 (Jamil et al. 2011).

In India, around 1.71 million Ha of land is affected by high salinity, of which about 72% of the area falls in Gujarat alone (cssri.org). Rann of Kutch is a saline wasteland in the state of Gujarat, located between 22°55’ to 24°35’ North latitudes and 70°30’ to 71°45’ East longitudes. The area is characterized by scant and seasonal rainfall and extreme temperatures. Rapid erosion through fluvial processes and capillary action has advanced the process of desertification by making the area saline with surface salinity ranging from a minimum of 281.6 ppm to a maximum of 12,094 ppm (Gupta and Ansari 2012).

Salinity impacts plant growth in myriad ways. Each developmental stage of plant such as germination, growth and reproduction is affected by salinity. Excessive amounts of sodium, chlorine, calcium, etc. can lead to ion toxicity. High sodium levels in the cell’s environment causes hyperosmotic shock and cell death (Munns 2002). Phosphorus is an essential element for plant growth and development. Excessive calcium ions cause precipitation of free inorganic phosphorus thereby reducing its availability to the plants. Reduced photosynthesis efficiency, nutrient deficiency, oxidative stress and excessive uptake of salts leading to nutrient imbalance are some of the ways salinity impedes plant growth. Growth of non-adapted flora in such soils is therefore impossible. This has led to vast stretches of lands rendered waste for cultivation purposes. This can be ill-afforded, given the fact that the continuously growing population, climate change and depletion of water resources are believed to severely hamper global crop productivity in not too distant future, resulting in widespread famine. Apart from reducing agricultural output, salinity disturbs the affected region’s ecological balance by limiting the diversity of micro- and macro-flora, impacts the physicochemical properties of the soil, causes soil erosion and influences the biogeochemical cycles (Hu and Schmidhalter 2002).

Despite low nutrient content and poor vegetation cover, hypersaline regions have recently been found to be populated by microorganisms adapted evolutionarily to survive and thrive in such environments, forming distinct community structures.
Gostincar et al. 2010). The organisms come from all three domains of life, namely, Archae, Bacteria and Eukarya. The presence of eukaryotes in hypersaline habitats, particularly fungi, was reported only at the onset of 21st century (Gunde and Cimmerman 2000). Since then, a number of fungal species and strains inhabiting natural hypersaline environments have been described. Based on cardinal range, these can be categorized as halotolerant, extremely halotolerant and halophilic (Kushner 1978). Halophilic fungi, a term coined for fungi that require some salinity for optimal growth, had previously been associated solely with foods treated with salt or sugar for preservation.

Fungal communities populating hypersaline environments have largely been isolated from the salterns along the Adriatic coast, the Mediterranean coast, the Red Sea coast, and the Atlantic coast, Gulf of California, Great Salt Lake, Utah and the West Coast of Indian peninsula (Gunde-Cimerman et al. 2005; Nazareth and Gonsalves 2014). These studies have yielded some important observations – halotolerant fungi are more prevalent in saline habitats rather than halophilic fungi (Hujslova et al. 2010; Nayak et al. 2012); halotolerant and halophilic traits are intrinsic characters of a particular species, rather than an adaptation in response to salt stress (Frisvad 2005); hypersaline environments do not harbor a characteristic fungal community of specialized taxa (Evans et al. 2013). The eukaryotic microbial communities in hypersaline environments are involved in ecological functions which include enhancement of sediment structures, decomposition of organic nutrients, and recycling of important elements (Park et al. 2004; Liu et al. 2014).

Survival of microbes in extreme habitats has necessitated biochemical evolution and the development of complex metabolic pathways, resulting in production of a diverse array of primary and secondary metabolites. Primary metabolites are produced by all living cells and are required for normal growth and functioning of cellular processes. Secondary metabolites are secreted only by a few members of the species, often a defining characteristic of the organism synthesizing those (Samson et al. 2014). Specifically, these are low molecular weight compounds, the secretion of which are linked to the organism’s morphological differentiation process, and are synthesized as families of related compounds (Zain 2013). Fungal secondary metabolites can be
broadly segregated into four chemical categories: polyketides (PKs), non-ribosomal peptides (NRPs), terpenes and prenylated tryptophan derivatives. Frisvad (2005) proposed that metabolites are often produced in highest diversity and amounts at up to 5% NaCl concentration, beyond which there is a steady decline in metabolite profile. There are two schools of thoughts regarding the metabolite production efficiency of an extremophile. Some are of the opinion that extremophiles, by virtue of the extreme nature of their growth environment, may possess complex metabolic pathways that yield novel metabolites. Whereas some believe that owing to low abundance of competing microorganisms, the metabolite diversity of an extremophilic microbe is limited. Although secondary metabolites are not essential for the organism’s survival, yet their genes being maintained consistently suggests that these compounds do provide critical benefits to the producer (Frisvad 2005). Some of their documented roles are tolerance against abiotic and biotic stresses, metal transportation, as sex hormones, differentiation effectors, agents of symbiosis between microbes and plants, nematodes, insects and higher animals (Wang et al. 1995; Demain and Fang 2000); many other roles are yet to be discovered (Omura et al. 2001; Bentley 2002; Zain 2013).

Sustainable agriculture is currently facing serious threats that are compromising food security for a rapidly increasing human population. Some of these challenges include the loss of usable land through overuse, deforestation and poor irrigation practices, which have led to desertification and salinization of soils, especially in dry lands (Helldén and Tottrup 2008). One of the approaches currently being adopted to face this situation involves development of stress-tolerant crops by genetic modification or breeding traits from wild plants. Crop genetic modification has generated a great public concern regarding their potential threats to the environmental and public health. Consequently, legislation of several countries has restricted their use in agriculture. On the other hand, exotic libraries from wild plants for clever plant breeding could overcome the problem of narrowed genetic variability of today’s high-yield crops. Plant breeding driven by selection marker has also been a major breakthrough (Breithaupt 2003). However, these approaches have met with limited success, probably because stress-tolerance involves genetically complex processes
and the ecological and evolutionary mechanisms responsible for stress tolerance in plants are poorly defined. New paradigms for sustainable crop improvement are currently arising. The above approaches do not consider the fact that plants in ecosystems have developed natural symbiotic associations for at least 400 million years with a broad diversity of microbial symbionts (Krings et al. 2007).

Exploitation of plant-fungal symbiosis appear as a smart alternative for plant adaptation due to their great quantity, ubiquity, diversity, and broad range of ecological functions they play in natural ecosystem. First coined by de Bary in 1866, the term ‘endophyte’ (Gr. *endon*, within; *phyton*, plant) refers to microorganisms living within plant tissues for all or part of their life cycle without exhibiting any visible symptoms of their presence (Bacon and White 2000; Tan and Zou 2001). Although all microbes exhibit endophytism, defined by Kusari and Spiteller (2012) as “a unique cost-benefit plant-microbe association defined by location that is transiently symptomless, unobtrusive, and established entirely inside the living host plant tissues,” the most common of these are the fungi. The first reports on endophytes appeared in the year 1898, wherein the presence of fungal mycelium was described in the seeds of *Lolium temulentum* (Darnel). Detailed studies were however carried out and reported by Freeman (1904) in his seminal work entitled ‘The Seed-Fungus of Lolium temulentum, *L.*, the Darnel.’ Ever since, researchers have isolated enormously diverse endophytes from an equally diverse range of plants.

Recent studies have shown that endophytes are crucial in the distribution of plant communities worldwide and are responsible of their adaptation to environments under high selective pressure (Rodriguez and Redman 2008). These indicate that some endophytes confer tolerance against specific stresses and are responsible for the survival of plants in hostile environments (Redman et al. 2002; Waller et al. 2005). An example of such habitat-specific adaptation has been reported in native grass species from coastal and geothermal habitats, which required symbiotic fungi for their tolerance to salt or heat (Rodriguez et al. 2008). Fungal endophytes help the host plants to withstand and tolerate the unfavorable environmental conditions like drought, high temperatures and salts (Malinowski and Belesky 2000). *Dichanthelium lanuginosum* is an herbal plant capable of growing in habitats where temperatures
reach up to 57°C. Colonization by *Curvularia* spp. renders the colonized plants the ability to withstand high temperatures and drought conditions. These beneficial effects were observed by systemically alternation in distal leaves, with increases the anti-oxidative capacity because of activation of glutathione ascorbate cycle in plants and results in grain yield.

It is currently thought that each plant in natural ecosystems comprises a community of organisms such as mycorrhizae and bacteria including endophytes. The ability of the endophytic fungi to confer tolerance to stress may provide a new strategy to mitigate the impacts of global climate change on agriculture and natural plant communities (Rodriguez et al. 2008). Such symbiotic lifestyles suppose a potential source for the improvement of food crops, through adapting them to situations of increasing desertification and drought on global crop lands. It appears, therefore, as a sustainable alternative to the use of genetically modified organisms. The plant-endophyte associations are strongly influenced by the specific soil type where they occur, which translates in an overall difference in species composition between communities from sand dunes and salt marshes. The major contribution to these differences is due to their dominant endophytes: *Fusarium oxysporum* for sand dunes or *Aspergillus fumigatus* and *Alternaria chlamydospora* for salt marshes (Rodriguez et al. 2008).

Groundnut (*Arachis hypogaea* L.) is an important oilseed and a major food crop cultivated throughout the world. In the year 2013, the global area under groundnut cultivation stood at 25.44 million Ha. India is the world’s second largest producer (4.69 million MT in 2012) and the largest exporter of the crop (0.749 million tonnes exported in 2011). It is the eighth most exported food crop of India, yielding about 932 million USD (FAO 2014). Groundnut is essentially a tropical plant, requiring a long and warm season and abundant sunlight. Sandy and sandy-loam soils at a pH between 6.0 - 6.5 are best suited for the plant’s growth. Groundnut is categorized as a crop sensitive to soil salinity, although the sensitivity varies with the crop variety. Salinity negatively impacts seed germination and seedling growth and dry matter production (Janila et al. 1999). Salinity also leads to Ca, K and Fe deficiencies in groundnut (Singh et al. 2004) causing yield losses (Hunshal et al. 1991).
The state of Gujarat is situated on the Tropic of Cancer and witnesses warm climes for the majority of the year. The geographical location and semi-arid nature make it an ideal region for groundnut cultivation. The chief roadblock in optimal utilization of the region’s land resources is that the majority of it is afflicted by high salinity. Wastage of such huge expanses of land can be ill afforded in a country like India stricken with a shortage of food crops. Given the high economic value of groundnut, its production is of immense importance for improving the socio-economic status of Indian farmers.

Halophiles are capable of successfully colonizing regions of high salinity, implying that they must possess a distinctive genetic and metabolic system imparting them this unique ability. In order to exploit the true potential of a halophilic fungus, in-depth studies are necessary to comprehend the organism’s growth and distribution patterns, biochemical architecture and its genetic makeup. A fungus’ chemoprofile is often a reflection of its genetic and biochemical makeup (Thrane et al. 2007). This property has consequently been employed in fungal chemotaxonomy, particularly for genera such as the Penicillium, Aspergillus and Fusarium and their perfect states, since these fungi produce consistent profile of secondary metabolites (Samson et al. 2014). A fungus’ chemoprofile also depends on the physicochemical nature of its habitat. For instance, fungi growing under low water index ($a_w$) produce a class of compounds known as polyamines that act as osmolytes (Laura and Jose 2014). Chemoprofile of fungi isolated from extreme ecological niches such as salt marshes and solar salterns are subjects of much interest as such fungi are expected to exhibit novel metabolic pathways and metabolites that are characteristic of that particular species or strain.

Microorganisms capable of successfully colonizing hypersaline habitats are well adapted to face chiefly two kinds of stresses – osmotic stress and ionic stress. Osmotic stress effects immediate dropping of cell turgor pressure, ultimately leading to cytoplasmic exudation and enhancement of intracellular solute contents. Inorganic ions are required for numerous biochemical functions. However, increase in their concentrations beyond a certain level leads to ion toxicity. For example, high NaCl contents inhibit enzyme activity and catalytic rate, disrupt membrane potential and cause protein coagulation among others. The cellular machinery of halophiles is
therefore optimized to perform two main functions: accumulation of organic solutes that do not inhibit cell’s functioning at high concentrations (the compatible solute strategy) and active extrusion of Na\(^+\) ions thereby maintaining the intracellular concentrations of Na\(^+\) at safe levels (Blomberg and Adler 1992). Other measures engaged by the halophilic organism to survive and thrive in hypersaline environments include modulation of plasma membrane, cell wall and fungal morphology (Kunčič et al. 2010).

Many different small molecules are known to serve as organic osmolytes and other compatible solutes. These can be carbohydrates such as sugars (e.g. trehalose), polyols (glycerol, inositols, sorbitol, etc.) and derivatives (such as o-methyl-inositol); amino acids (glycine, proline, etc.) and derivatives (e.g. ectoine).

Trehalose is a nonreducing disaccharide possessing a unique α, α-1, 1 glycosidic linkage and is therefore unreactive. An energy and carbon reserve, trehalose plays an important role in fungal development and metabolism, contributing to energy requirement in cell processes such as glycolysis, sporulation, and germination. Trehalose-deficient mutants of many fungal species exhibited significant developmental perturbations. Trehalose also functions as an osmolyte; an increase in trehalose synthesis in response to abiotic stresses has been reported in a wide variety of organisms, ranging from yeasts and nematodes to higher plants, where the compound has played protective roles such as membrane stabilization and protein preservation under a wide range of stress conditions such as temperature shock, desiccation, radiation and oxidative stress (Elbein et al. 2003; Wyatt et al. 2015). The compound surpasses other solutes with respect to its protective properties (Kaushik and Bhat 2003). Studies conducted to determine the role of trehalose in *Saccharomyces cerevisiae* subjected to osmotic stress have established the compound’s role as an osmoprotectant (Hounsa et al. 1998; Mackenzie et al. 1988). The compound reportedly binds to macromolecules and membranes and replaces water molecules thereby maintaining their basic structures.

Polyamines are a group of aliphatic polycations found in both prokaryotes and eukaryotes. Putrescine, spermidine and spermine are the most common polyamines
present in higher eukaryotes. However, most fungal species lack spermine. In fungi, polyamines are involved in cell proliferation and differentiation processes such as spore germination and conidiation and filament formation (Herrero et al. 1999). Low polyamine levels in cells result in growth inhibition, whereas its excessive levels lead to cytotoxicity (Igarashi and Kashiwagi 2010; Hu and Pegg 1997). Inhibition of putrescine synthesis inhibits sporulation and spore germination (Ruiz-Herrera 1994). Polyamines have been suggested to be involved in osmotic stress adaptation by maintaining cellular homeostasis. For instance, survival of *S. cerevisiae* in conditions of high salinity coincided with accumulation of polyamines, suggesting that polyamines are involved in maintaining cellular homeostasis under saline stress (Lee et al. 2002; Aouida et al. 2005).

Enhancement of intracellular glycerol content has been a standard feature of the microorganisms under saline stress. In numerous studies conducted on the cellular response to salt stress, glycerol has emerged as the primary osmoticum (Tekolo et al. 2010; Kogej et al. 2007; Janja et al. 2014). Microorganisms can elevate intracellular glycerol levels either through enhanced synthesis during osmotic stress or up take from the surrounding environment or through greater retention in the cytoplasmic matrix. Glycerol as an osmolyte cannot be substituted by other polyols such as sorbitol or mannitol, as the compound is supposedly involved in lowering of NADH, in addition to osmotic adjustment.

Osmotic stress adaptation strategies have been elucidated predominantly in the salt sensitive *Saccharomyces cerevisiae*. However, in order to truly understand osmoadaptation mechanisms, the extremely halotolerant black yeast *Hortae werneckii* and the most halophilic fungus *Willemia ichthyophaga*, are considered as suitable model organisms. *Hortae werneckii* is an extremely halotolerant black yeast which grows optimally between 0.8 M and 1.7 M NaCl, and is capable of growing up to saturated NaCl. *W. ichthyophaga* is the most halophilic fungus reported to date. It has an optimal growth salinity of 15-20% NaCl and the capability of growing up to saturation (Gunde-Cimerman et al. 2000; Gunde-Cimerman et al. 2014). Other organisms on which halotolerance studies have been conducted include the polyextremotolerant *Aureobasidium pullulans* and the marine yeast *Debaromyces*...
hansenii (Gunde – Cimerman et al. 2000; Managbanag et al. 2002; Kogej et al. 2005; Kogej et al. 2007). These fungi adapt to high osmotic stress by accumulating compatible solutes. The polyols content in the above mentioned fungi was found to be at elevated levels, with glycerol being the main polyol. The presence of other polyols such as mannitol and arabinol were also detected.

Accumulation of compatible solutes alone is not sufficient to counter salt stress. Excessive concentrations of Na$^+$ in extracellular environment cause its rapid influx, leading to Na$^+$ toxicity. A low Na$^+$/K$^+$ ratio is essential for salt tolerance. Kogej et al. (2005) demonstrated that H. werneckii maintains very low intracellular Na$^+$ and K$^+$ levels by exudation of Na$^+$ ions. Similar results were reported in S. cerevisiae. On the contrary, Na$^+$ levels were reportedly high in W. ichthyophaga growing at high salinities, suggesting that the fungus could be a Na$^+$ include organism (Kunčič et al. 2010). Similar observations were made in D. hansenii, although high Na$^+$ content eventually caused reduction in growth (Prista et al. 1997).

Growth at high osmotic stress also entails modulations in the cell morphology. The fungal cell wall acts as first line of defence against a host of abiotic stresses. Apart from imparting mechanical strength, the cell wall is also necessary for the normal functioning of the cell. An organism’s survival under abiotic stresses such as hypersalinity, therefore, depends on the state of its cell wall. Thickening of cell walls has been observed in fungi such as those belonging to genus Wallemia, Trimmatostroma and Dendryphiella, when subjected to high salinity, suggesting that the thickened cell wall is essential for survival under osmotic stress (Kunčič et al. 2010). Shortening and thickening of hyphal compartments have been observed in the halotolerant (for ex. Aspergillus repens) and halophilic (for ex. W. muriae and W. sebi) fungi growing in high salinity. In the most halophilic fungus, W. ichthyophahagia, sarcina-like structures were observed at high salinities (Kunčič et al. 2010). Strengthening of cell wall can be effected by increase in chitin concentration, enhanced β-1,3-glucan concentration, degree of branching of polysaccharides or incorporation of proteins into the cell wall (Kapteyn et al. 1999; Klis et al. 2002; Lesage and Bussey 2006).