Chapter 10
Systemic Approach in Determining the Role of Bioactive Compounds

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Abstract Biosystems theory provides a useful framework for describing the biological role of bioactive compounds. Each functioning biosystem must adapt to and, to a certain degree, possess mechanisms or means to control both intra- and extra-organism conditions, "*purpose*". The organism must have the capabilities to change itself or the external environment, including community function, to achieve a purpose at the highest possible level. In general under adverse conditions, an organism is forced to renounce the purposes of higher level and to fulfill the purposes of a lower level. The achievement of higher level purposes depends on the production of natural bioactive compounds (adaptogens). The role of bioactive compounds is discussed herein from the point of general concept of biosystems, their resistance, and their adaptation to adverse conditions. Utilization of the concept of a biosystem will provide a clearer understanding of the role of bioactive compounds in the sustenance of an individual redox state and to predict new functions and properties of the organisms and their bioactive compounds.

10.1 Introduction

For many years, humankind has benefited from the use of green plants and animal products as sources of drugs and herbal remedies. Natural drugs (e.g., antibiotics) derived from microorganisms have a much shorter history of exploitation to improve human health. Their major impact on medicine goes back only about 60 years to the

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introduction of the antibiotic penicillin to control communicable diseases. Microbial produced antibiotics now account for a very high proportion of the drugs commonly prescribed. Animal related drugs, such as steroids and prostanoids, are also in large usage. With time, the concept that the exact boundary between remedies and food products does not exist is emerging. One of the confirmations of this observation is the recognition that some products, for example from fungi, concomitantly represent pharmaceuticals and health foods. It is also well known that the quality of plants, animal, and microbes as source of medicine and food depend on conditions of habitat or cultivation.

Two primary areas of interest that are emerging relating to these biological products are a elucidation of their role in the general metabolism of the individual organism and the importance of these substances to the functioning of the biological community considered as a whole. These studies have led to the development of a general theory of biosystems development and their environmental interactions, which is outlined in the current article.

10.2 Concept of Biosystem: The Basic Properties of Biosystems

Each biological object represents a biosystem in itself in that the intracellular organization of less complex organisms as well as the complex of organs comprising higher organisms necessitates specific controlled interactions of the organisms' components [1]. To simplify our discussion, the levels of biosystems could be divided into three categories: the *under-organism level* (intra-organism interactions) – cells, tissues, organs; *the organism itself*, and the *super-organism level* – (inter-organism interactions) – population, ecosystems, and biosphere. It is clear that each biological entity is a concomitant element of the next higher level system as well being a system unto itself *per se*. The higher level systems integrate all of the processes of the lower level systems contained therein into a communal whole; directly and indirectly, regulating or influencing all inferior lower levels of integration [2]. A necessary consequence of this multilevel interaction, that with each new level of structural and functional interaction, new functions must appear, which are frequently impossible to be predicted based on simple observations of the activity of the various lower-level components. As a result, when analyzing the properties of a biosystem, it is necessary to explore not only the links at the same level (e.g. *processes of development with time*), but also the vertical interdependence of phenomenon at different levels of integration.

A primary concept essential for understanding the function of biosystems is the fact that they are open systems [3]. The existence of this open system impact nature and quantity of its products as well as various aspects of energy assimilation, accumulation, transmission, and utilization, all associated with ensuring maintenance of the biosystem structure, its growth and function. *The second* side of function is related to orientation of energetically processes. It includes perception, storage,

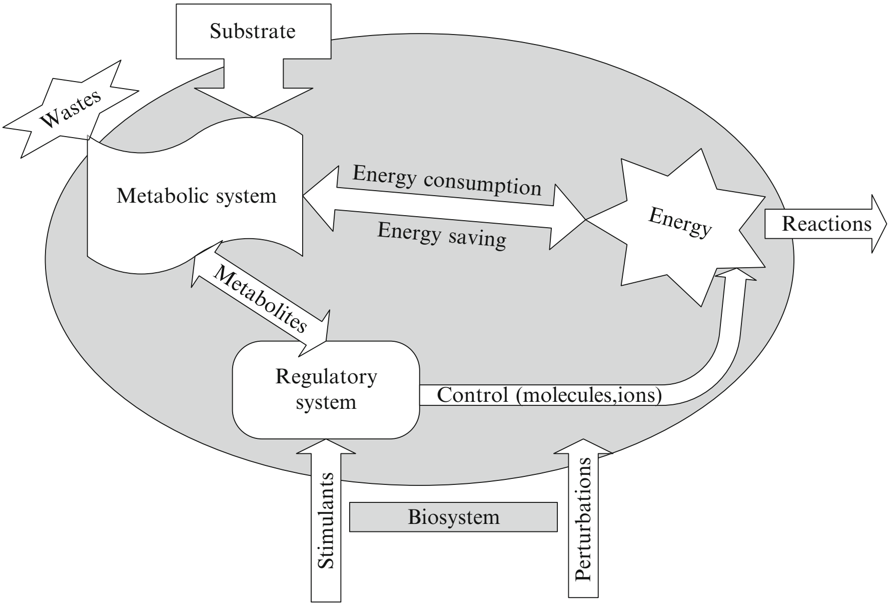

Fig. 10.1 Substrates, energy, and information flow in biosystems. See details in text

processing, and utilization of information. The mechanisms that handle information determine the nature of active energetic process and their rates in the system. This aspect of function is responsible for determination of the rate and direction of biosystem development and adaptation.

A depiction of energy and information flow in relation to system function is provided in Fig. 10.1. The energetic component is designation as *metabolic system* (***MS***); the operational portion of the process is presented from the view of regulatory system (***RS*** – *genetic and physiological regulation*), and blocks of the effectors. In this schematic, two the main characteristics of biosystems are demonstrated – nutrient exchange (*the opened portion of the biosystem*) and process control.

The anticipated final state of a biosystem, which is primarily the product of its function or a final structural status achieved in consequence of its physical organization, has been termed the "*the purpose mechanisms of handle*". In accordance with the theme of the workshop, we discuss only the components of "*purposes*" that ensure life through provision of all necessary metabolic and energy resources (agents) and with protection of life functions from the damaging factors of the organism's environment. *The first aspect* of *maintenance capacities* of biosystems is related to insurance of constant internal conditions necessary for sustenance of "life", *homeostasis*. *The second aspect* of maintenance of biosystems derives from the necessity to provide adequate acquisition of nutrient and energy resources from sites outside of the organism itself. This assures the *stationary non-equilibrium state*. The stationary non-equilibrium status between processes of input and output of substances and energy is characteristic for all living systems, but only under conditions of homeostasis are they at optimal (economical) levels.

Sustainability of biosystems can conveniently be discussed in the terms of *flow rate* and *levels* [4]. When describing living systems, we are operating at two levels. *Firstly*, the content of energy and metabolic resources within and outside the biosystem must be considered. The variables to be considered in this aspect are termed, *levels*. *A second level* of consideration involves evaluation of the flux rates of these substances, such as the dynamics of accumulation, uptake, assimilation, recycling and excretion of substances. These are designated as process *rates* – rates of enzyme synthesis, rates of growth, rates of oxygen utilization etc. The achievement of a reasonably constant internal environment requires maintenance of the level of nutrient resources and energy in the interior of the system. Biological regulatory systems, essential in maintenance of the internal and external homeostatic conditions, are in generally directed to achievement of specific essential cellular or organismal functions – i.e., to maintain a *highly efficient level of cellular function* – effective energy and nutrient consumption, economical or efficient utilization of nutrients, and reliable or sustainable function [5].

In Fig. 10.1, the relationships are depicted as *metabolic system, regulatory system*, and *energy*, associated with intra-system level functions. External components are influenced by the intra-system reactions and as well by the intra system activity due existence of signals from interior of this system (waste, reactions, redox state). In biosystems with different levels of organization (microorganisms, higher organisms, populations, ecosystems, and biosphere) external and internal components are of varying complexity. For example, in an RS within the organism activity results from individual genetic capability, but in the community – it necessarily is the product of the genetic capability of different members of the community. That is, biosystem of higher complexity include external signals of all biosystems of which they are composed.

Under adverse conditions, the biosystem must adapt to its environment, as allowed by the capability contained within its genetic makeup, to modulate internal and external functions and to induce modification of the external environment. For example, exhaustion of nutrient and energy resources must necessarily result in a diminution of the level of function of the biosystem. In physiological terms, initially the system is subjected to the strain induced by overall physical and chemical limitations of the system, but subsequently as required resources are diminished or natural conditions degraded, a situation that could be described as pathology occurs [6]. In contrast to the normal situation, that assure optimal conditions for life, the organism must now expend energy and resources necessary to attempt to restore homeostatic conditions in a "pathological" environment. In Fig. 10.2, a hierarchy of three purposes of biosystem under influence of two factors, described with two variables, V_1 and V_2, is shown schematically. A large range of conditions (S_1) exists where the organismal or cellular regulatory mechanisms are capable of assuring a reasonably constant range (*the regime of stationary non-equilibrium state*) of biosystem function. The area (S_2) of more favorable conditions, where the biosystem is capable of maintaining the homeostasis, is more limited. Lastly, we could designate

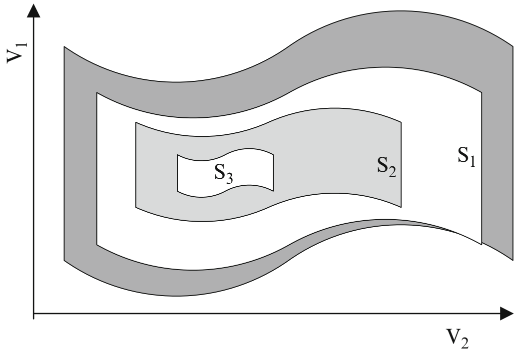

Fig. 10.2 Hierarchy of biosystems' purposes

a more limited range of conditions (S_3) where optimal organismal function occurs. As the most favorable conditions for biosystem functioning are degraded, the parameters of conditions exist outside the narrow region (S_3) and biosystem transitions to a lower level of function (in region S_2). Further degradation of the organism's environment may result in its having to function further from the region S_2 (where biosystem is able to maintain homeostasis). Additionally situations may easily be described where conditions are for region S_1 and even outside this region. Outside the conditions marked by region S_1, the biosystem can only be maintained alive for a limited time period. In Fig. 10.2 this region is designated outside the area S_1. It is important to note that the requirements for conditions of environment that are sufficient for maintaining the biosystem at lower levels of function are necessary, but not sufficient for maintaining them at higher levels. This means that to maintain viability under adverse conditions, the biosystem gradually abandons the higher level purposes and transfers to maintaining the purpose of lower level. Under stressful conditions, the biosystem does strive to achieve the highest possible purpose. When conditions become favorable, the biosystem gradually pass from purpose of lower level to those of higher levels.

From a practical perspective is extremely important to determine the appropriate parameters that indicate the transition of biosystem from one state to another (conditions of stress). This would enable to compare the stress resistance of different biosystems, and as well the influence of various factors on their resistance and as well the recovery of damage caused by stress. An indicator of the biosystem resistance could be the redox state, but it is necessary to consider that in the complex biosystems it can be at different levels in different compartments. As all provoked by stress damages and their recovery may be specifically in biosystem components, processes must be classified in accordance to their levels of manifestation. Figure 10.3 demonstrates the possible ways of recovery of biosystems response to stressful conditions. Under extreme stressful conditions, the response of the biosystem may be the result of many complex interactions, some of which are to distinguish without supplementary experiments. The existence of various mechanisms to withstand stresses reflects the capacity of biosystem to abandon (gradually, in conformity with the degree of aggravation of environmental conditions) the hierarchically less important purposes required for maintaining life.

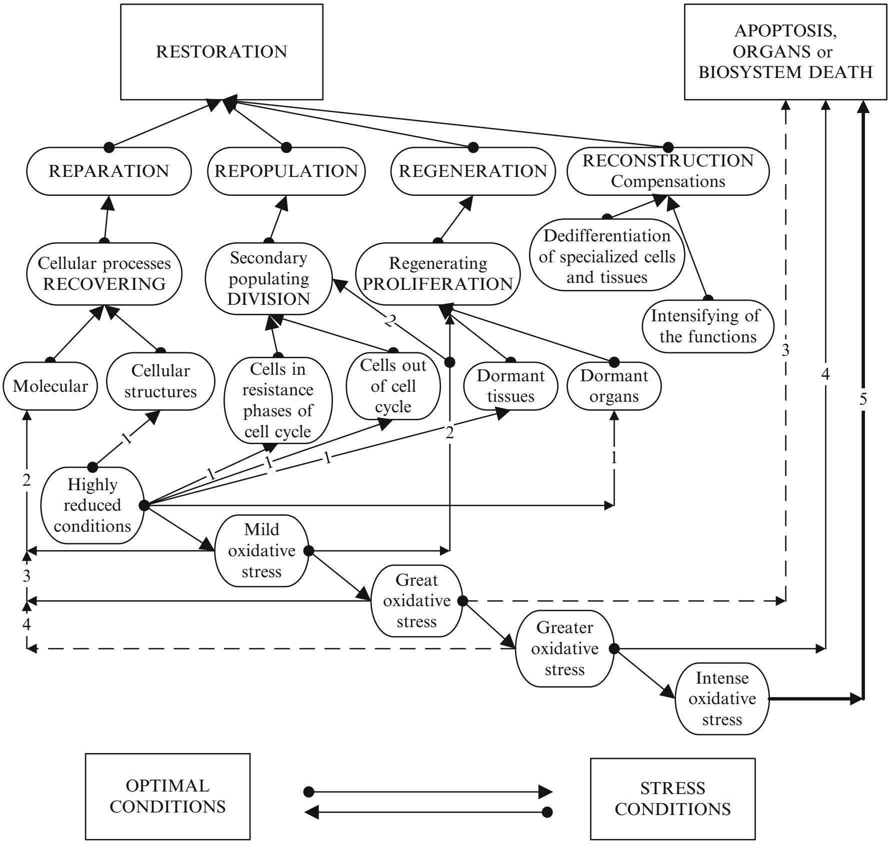

Fig. 10.3 Change of status and possible routes of biosystem recovery in response to stress factors [7]: *1* – highly reduced conditions, *2* – mild oxidative stress conditions, *3* – great oxidative stress, *4* – greater oxidative stress, *5* – intense oxidative stress

Recently much has been made to elucidate the molecular components common for resistance. It was demonstrated that there are several common ways of controlling the plant response to different stressors. The first adjustment is made involving transcription a complex set of interacting signals pathways and provides common and specific induction of resistance. Rate genes encoding proteins involved in biosynthesis of transcription factors is about 25 % [8]. The stress-response relationships illustrated in Fig. 10.3 show the changes in stress conditions of the flow in substrates, energy, and information in biosystems illustrated in Fig. 10.1. The factors that act at early stages of stress response are critical for other biosystems functions. They use common pathways and components in the stress-response relationship. This makes possible cross-tolerance, concomitant adaptation/acclimation to different stresses after exposure to one specific stress. Responses to stressors are based on the general changes that occur under the influence of stress. One of such changes is dehydration. Elements that respond to dehydration and genes that are regulated by these factors were detected in plants [9]. The activity of transcription factors may be related to the influence of hormones and determined the differences in resistance to stress of related species. A single transcription factor can coordinate the expression of many genes to improve stress tolerance.

It is worth noting that, in many cases, plants survive stress by metabolic arrest, in which growth and development essentially stop. The common response of plants to different abiotic and biotic stresses, such as heat, drought, cold, high-light intensities, wounding, UV, ionising radiation, ozone, and pathogens is the accelerated production of *active oxygen species* (AOS). Among them the most important are hydrogen peroxide, hydroxyl radical, and the superoxide. The action of H_2O_2 as a signal in the induction of different catalase genes has been shown [10]. The peroxidases also control the amount of H_2O_2 present within the plant cell and its concentration reach the level that trigger death only at intense oxidative stress (Fig. 10.3). H_2O_2 is a local and systemic signal in plants adaptation to high light, tolerance to heat shock and low temperatures, growth responses to environmental stimuli [10]. This indicated the important role of the H_2O_2 in induction of the cross cores tolerance and in the stress survival network. Changes in H_2O_2 homeostasis are representing signalling event and induce the enhancement of stress tolerance. When the oxidative stress is intense, high concentration of H_2O_2 may leads to programmed cell death. H_2O_2 interacts with other signalling systems, particularly hormones, modify the action of other secondary messengers such as Ca^{2+} and NO, and act via modification of signal transport system in optimal and stress conditions.

10.3 Bioactive Compounds and Their Role in Biosystem Resistance and Adaptation

Cell processes are regulated also by oxidation and reduction, but phosphorylation and dephosphorylation is equally important. The redox state of the cell influences phosphorylation, and vice versa. Some kinases can be directly and indirectly affected by AOS. Balance between AOS and antioxidants are important. The bioactive compounds (and adaptogens) acts directly as antioxidants, or in conditions of stress have beneficial effects changing the antioxidant – prooxidant balance in favour of the former, leading to potential recuperation. Increased oxidative damage can result not only from more oxidative stress, but also from failure to repair or replace damaged biomolecules.

It is generally concluded that bioactive compounds influence an organism's health and resistance to different adverse environmental stresses. An important step in understanding the nature and mechanisms of their influences on biosystems appeared after introduction in 1947 of the concept of adaptogens by Lazarev et al. [11]. They were defined as substances meant to put the organism into a state of non-specific heightened resistance in order to improve resistance to stresses and to adapt to extraordinary challenges.

As in the case of stress, the conception of adaptogens was initially developed to explain the protective effects of some natural products on human and animal systems and was later extended to include their effects on all biosystems. There are well known parallels between the action of some substances and occurrence of disease resistance in plants and animals. The most impressive example of such substance is salicylic acid (SA) and its derivatives, isolated from extracts of different plants. It appears to have multiples modes of action since exert a wide range of clinical effects including reduction of pain, fever, inflammation, blood clotting, and the risk of heart attacks and strokes. Exogenous supplied SA has been shown to affect a large variety of processes in plants, including stimulation of stoma closure, seed germination, fruit yield and glycolysis. Adaptogens help biosystems to promote the capacity to rapidly adapt at the cellular, tissular and organism level to different stress factors in process termed Non-Specific Resistance Stimulants NSRS (for animals) and Systematic Acquired Resistance SAR (for plants) [12, 13]. The areas of the adaptogens benefits are stress protective action, activation of immune system, improvement of mental and physical work capacity, normalizing effects, antioxidant and anti-aging action, augmentation of performance, endurance and rehabilitation etc.

Unfortunately, one very essential feature of adaptogens is not fully discussed in the scientific and population literature: that is, their effects are realized at very low concentrations. It is known that mechanism of action of adaptogens depends on many factors, including chemical properties of adaptogen, genotype specificity, and age of organism, stage of development and even season of the year [1]. In our opinion, the different effects of bioactive compound on biosystems are a useful consideration from the view of general theory of biosystem, resistance, stress, and adaptations. This type of analysis could be very important because it is necessary to generalize the causes of existence of reduced number of stress reactions to different stress factors of organisms that belong to different kingdoms. Generally speaking, the beneficial effects of adaptogens could be due to enlarging one or concomitantly many areas (S_3, S_2, and S_1) indicated on Fig. 10.2.

Although a detailed analysis of mechanisms of action of adaptogens is beyond the scope of this article, it could be useful to demonstrate the efficacy of utilizing the systemic approaches in consideration of the role of heat-shock proteins (hsp) [14] in adaptation. The synthesis of these proteins, in prokaryotes and eukaryotes, is induced during a stressful event, such as high temperatures. Additionally, many of the "*stress proteins*" play important roles for normal cellular functions under stress-free conditions, especially in periods of development, differentiation and growth (influencing on recovery of molecular and cell structures (Fig. 10.3). The prompt induction of hsp in stressful situations is a vitally necessary protective function for protection of sensitive cell proteins from denaturation, recovery of damaged protein and reparation of cell structures (Fig. 10.3). They also influence RNA and protein-synthesis, temporarily inactivate certain receptors or initiate immune reactions that will influence repopulation, regeneration, and reconstruction (Fig. 10.3). These effects of *hsp* are extending of the physiological adaptation; protect energy resources from depletion, and accelerate the biosynthesis of proteins and nucleic acids.

A specific example of the adaptogen effect is the experimental results from study of a preparation Reglalg (Fig. 10.4). This preparation includes a mixture of

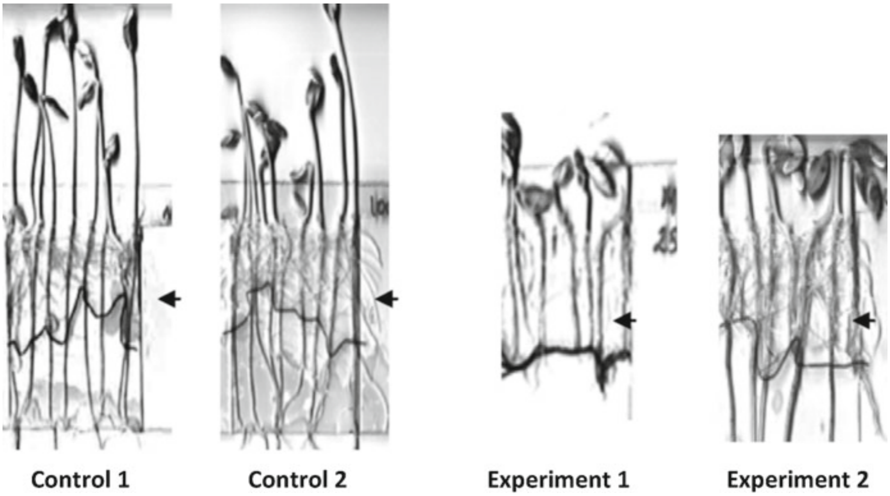

Fig. 10.4 Photos of 7-day old cucumber plantlets obtained from the seeds untreated (Control 1 and Experiment 1) and inoculated with a solution of preparative Reglalg (Control 2 and Experiment 2). Three days seedlings of Experiment 1 and Experiment 2 were treated with heat shock (5 min emersion in water with temperature 45 °C). The length of the roots at the time of heat shock is indicated by the *dark lines*, as emphasized with the *arrows*

unsaturated fatty acids, aldehydes, ketones, aldehyde-ketones and other bioactive components, prepared from algae in special conditions. Successful combination of such components assures a wide spectrum of applications. Plantlets of *Cucumis sativus* obtained from the seeds sprinkled with the preparation Reglalg (Control 2) had a small tendency to produce more developed roots system and less developed shoots in comparison with those obtained from seeds not treated with Reglalg. Under the influence of heat shock the growth of the roots of the plantlets obtained from seeds untreated with Reglalg was stopped completely (Experiment 1) and those obtained from seeds sprinkled with Reglalg continue to grow at the level comparable with that of control plants (Experiment 2). The preparation Reglalg could be considered to be an adaptogen. Under its actions the resistance of plants to heat shock increased (the area 2 of value of parameters of homeostasis maintaining became larger – Fig. 10.2). In supplemental experiments, it was shown that the beneficial effects of preparation Reglalg was partially dependent upon its protective action increasing resistance and as well due to cellular and molecular events immediately after heat shock (Reparation and Repopulation, Fig. 10.3). Thus the system theory helps biological researchers in analyzing a complex experimental results or designing new experiments.

From previous discussion, it is apparent that utilization of bioactive compounds by biosystems could be regarded as external signals (Fig. 10.1). The effect of each external signal is determined by the "target" of its action. If it influences the efficacy of MS, the product could be regarded mainly as a nutrient. If it activity is limited to RS, this products is a medication or adaptogen. Most obviously the bioactive compounds influence both the MS and RS and act concomitantly as a nutrition and medication. It is important to state that the action of bioactive compounds also depend from the biosystem at which it influences. If this biosystem consists of a single organism, the influence could be as nutrition or/and medication. In the case of population or community in the biosphere, the impacts are primarily at the ecosystem level. Chemicals with antibiotic, insecticide, or even chemoattractant properties provide excellent examples of byproducts produced to improve the status of individual members of a community that may also directly or indirectly impact humans or be adapted to improve human health or food production. The widespread exploitation of biologically produced antibiotics discussed earlier in this presentation provides a prime example. A group of very interesting antibiotics that affects both plant microbe interactions as well as iron nutrition are siderphores. They are produced by bacteria and plants and provide an excellent example of substances affecting biosystem productivity through metabolic enhancement in that they are instrumental in controlling iron availability to both bacterial communities as well as higher plants [15, 16]. Additionally, these substances can be utilized to provide biocontrol of plant pathogens [17]. Another example of soil bacteria enhancing plant biomass production involves plant hormones. Soil microbes growing in the rhizosphere are a source of plant hormones [18]. The benefits of the hormones to the plant are clear, but the gains provided to the soil microbes are less obvious. The rhizosphere microbes benefit from this seemingly altruistic action by the fact that the plant is their primary nutrient and energy source. Thus, by stimulating plant biomass synthesis, the microbes gain an enhanced nutrient and energy resource.

Additional examples of bioproducts in maintaining extracellular homeostatic conditions would be production of natural pesticides by higher plants. A particularly interesting example of the latter is the production of pesticides by grass containing fungal endosymbionts [19]. These are a few examples of the pool of potentially useful bioproducts synthesized by plants, microbes, and animals that may be exploited to improve crop productivity and human health. These few examples provide evidence of the diversity of biological substances produced that control community structure, productivity and external threats. New interactions are continually being discovered, including some of the more recent studies of quorum sensing substances in biofilms, etc. From these limited examples it is clear that much more remains to be learned about the diversity and utility of bioactive compounds. Approaches discussed in this presentation are useful to classify and determine the potential role of mentioned substances as food or adaptogens for interacting biosystems of different level.

10.4 Conclusion

1. The biological functions of bioactive compounds as food, medicine and determining ecological equilibrium overlap considerably.
2. Implementation of the conception of biosystem and systemic approach is very useful in: (a) determining the “targets” of the influence of bioactive compounds on different levels of biosystems; (b)understanding the specificity of influences

of stress conditions on biosystems and changes of their purposes in dependence of stress level and duration; (c) elucidating the relative influence of bioactive compounds on biosystem as food, adaptogen and agents that determine ecological equilibrium; (d) elaborating effective systems of screening of bioactive compounds with adaptogen and ecological functions; (e) determining the biosystems resistance to different stress factors by appreciating the changes of redox potential under exposition to stress at different doses.

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