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ORIGINAL ARTICLE

Somatic embryogenesis and plant regeneration from leaf explants of endemic *Begonia pavonina*

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ABSTRACT

An efficient protocol for the regeneration of endemic *Begonia pavonina* plants via somatic embryogenesis was developed. Somatic embryos were induced from *in vitro* leaf explants cultured on Murashige and Skoog (MS) medium supplemented with 1.0 and 2.0 mgL^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with 1.0 mgL^{-1} of 6-benzylaminopurine (BAP). Histological examination successfully revealed all stages of somatic embryos; globular embryos, torpedo and heart-shaped structure were induced from callus. Approximately 90% germination for development of somatic embryos into complete plantlets were achieved, upon subcultured onto MS basal medium. This is the first report of somatic embryogenesis in *B. pavonina* with significantly high plantlet regeneration frequency.

Key words: Endemic; *Begonia pavonina;* Somatic embryogenesis; MS medium; 2,4-D; BAP; Histology.

INTRODUCTION

Begonia belongs to a family of Begoniacea. *Begonia pavonina* is a plant species that endemic to Malaysia, and is only known to be found in the Cameron Highland (Malaysia) area. *B. pavonina* is also known as the Peacock Begonia and is one of the most remarkable Begonias in Malaysia because of its leaves, which are like peacock feathers, change colours from iridescent blue to bright green, caused by refraction of light [1]. Begonias are grown as decorative houseplants and for landscaping. Over 10 000 Begonias hybrids and cultivars have been introduced by commercial growers [2, 3] and new species continues to be discovered and described [4]. Among some of the commercially important species are *B. sempenflorences, B. tuberhybrida, B. elatior, B. cheimanta* and *B. socotrana* [5].

Since it is an important ornamental plant throughout the world, commercially large-scale propagation of these valuable Begonias are considered necessary, to meet the ever increasing demand. Tissue culture techniques have long been used to produce thousands of high quality seedlings instead of the traditionally used cuttings. Plants production through *in vitro* culture is essential to safeguard the sustainability of this endemic

B. pavonina. Plant tissue culture technology has been extensively used to propagate a number of begonias all over the world such as *B. cheimantha* [6-8], *B. erythrophylla* [9], *B. rex* [10], *B. tuber-hybrida* [11, 12], *B. franconis* [13] and *B. simper-florens* [14].

Propagation through somatic embryo-genesis has been reported in a wide range of plants. Somatic embryogenesis has several advantages over organogenesis and seedling production, including reduced proliferation time durations, in which the plantlets were formed in fewer steps and cytological uniformity of the plantlets [15]. Plant regeneration from somatic embryogenesis has higher genetic integrity over the traditional organogenesis [16-18], thus this technique is the most desirable approach due to genetic stability of regenerated plantlets [19]. In addition, somatic embryogenesis offers valuable tools for genetic enhancement of commercial crop species [20], and possibility to produce artificial seeds and germplasm conservation by cryopreservation [16, 21].

Although somatic embryogenesis of few begonias have been established [3, 22] to this date, no report is available for *B. pavonina*. Thus, somatic embryogenesis of *B. pavonina* from leaf explants *in vitro* was reported for the first time in this paper. Histological examination on various stages of somatic embryogenesis is also being discussed. The development and germination of *in vitro* plantlets derived from somatic embryos were also explained.

2. MATERIALS AND METHODS

2.1. Plant materials and explant preparation

B. pavonina wildings were collected from Cameron Highlands, Malaysia and maintained in FRIM's nursery. Young leaf was surface sterilized with 70% (v/v) ethanol for 30 sec. followed by rinsing with sterile distilled water (SDW) for several times. The explant were then immersed in 10% (v/v) sodium hypochlorite with a drop of Tween 20 for 20 min and rinsed with SDW. The explants were cultured onto MS [23] basal medium supplemented with 0.1 mgL^{-1} BAP for shoot development. The plantlets were maintained at 25 ± 2 ^{0}C with illumination powered by cool-white florescent light (16-h photoperiod) and further subcultured every 4 weeks.

2.2. Induction of callus and somatic embryos

In vitro grown plantlets derived after the third subculture were used as an explants. Four *in vitro* leaf segments with three replicates using a completely randomized design were cut into approximately 1.0 cm^2 and cultured onto MS medium supplemented with different concentrations of 2,4-D (0.5, 1.0 and 2.0 mgL^{-1}) with or without combination of 1.0 mgL^{-1} BAP for embryogenic callus induction.

Leaf segment cultures were maintained in continuous darkness with temperature 25 ± 2 ^{0}C. Subculturing onto fresh media was routinely performed (every four weeks) until callus appeared on the explants. Friable embryogenic callus, identified by its creamy white colour was cultured onto fresh MS medium to accelerate the induction of somatic embryos. Morphological development of somatic embryos were observed under a binocular microscope weekly.

2.3. Regeneration of plantlets

Somatic embryos from various stages (globular, heart-shaped and torpedo shaped) were separated and transferred individually into MS basal media for plant regeneration. The cultures were maintained under ambient growth room condition (25 ± 2 ^{0}C, 16-h photoperiod and *ca* 45% relative humidity). The development of plants were observed weekly.

2.4. Histological studies

Histological examination was done according to protocol established by Malaysian Palm Oil Berhad, Malaysia. Samples of different stages of somatic embryos were fixed in EAF Fixative Mix (50 ml absolute ethanol, 5 ml acetic acid, 10 ml formaldehyde 37% and 35 ml of distilled water) for two days at 4 ^{0}C. The tissues were washed, dehydrated through an ethanol-xyelene series and embedded in paraffin wax. Tissues were sectioned with 10 µm thickness using a rotary microtome (Leica RM2165 Microtome, Germany). Tissues were mounted with Cytoseal^{TM} 60 Mounting medium (Stephens Scientific, USA) and stained with Schiff's reagent and Naphthol blue black.

Tissues were photographed using laser camera system (Jenoptic Prog Res C10plus, Germany).

3. RESULTS

3.1. Callus induction

Callus formation was observed on the cut edges of the leaf explants within two weeks on MS media tested. On the subsequent weeks, most of the leaf segments started to expand in size and formed a cluster of small whitish callus clusters on the margin as well as on the cut surfaces of the explants (Figure 1B). After 4 weeks of incubation, callus mass increased and covered the entire surface of the explants (Figure 1C). Callus was mostly friable and whitish–yellow in colour. These calli were detached and sub-cultured onto their respective fresh media every four weeks; thus initiating new, creamy and compact to semi-friable callus.

Small and translucent shoots germinated on the callus tissues on media supplemented with 2,4-D (0.5 mgL^{-1}) and 1.0 mgL^{-1} BAP; suggested that inclusion of BAP in lower concentration of 2,4-D would favour shoot organogenesis.

Callus proliferated on MS media supplemented with 2,4-D alone were compact and yellow in colour. This type of callus failed to form either embryogenic callus or shoots, and did not respond to further subculturing onto fresh media, hence, not suitable for inducing somatic embryogenesis.

3.2. Induction of somatic embryos

Higher frequencies of callus induction from explants were obtained on the MS media supplemented with higher concentration of 2,4-D (1.0 and 2.0 mgL^{-1}) in combination with BAP (1.0 mgL^{-1}). After 6 weeks of incubation on the same medium, granular and shiny masses of callus were observed. This process continues in the subsequent subcultures leading to the successive formation of somatic embryos that emerged from the peripheral areas of the callus mass after 8 weeks of culture incubation. Various developmental stages were observed. The somatic embryos first appeared as clusters of globular embryos (Figure 1D). Subsequent subcultures leading to further development into torpedo and heart-shaped structures after 12^{th} weeks of incubation (Figure 1E).

3.3. Germination of somatic embryos

Well developed embryos were dissected individually and transferred onto fresh MS basal medium for plantlet regeneration. Most of the embryos germinated and developed into complete plantlets within 2-3 weeks (Figure 1F).

3.4. Acclimatization of tissue culture raised plants

Well develop plantlets were transplanted on sand medium with percentage of survival approximately 90% (Figure 1G).

3.5. Histological examination of somatic embryos

Histological examination confirmed the induction of somatic embryos directly from *in vitro* leaf explants on MS medium containing 2,4-D (1.0 and 2.0 mgL^{-1}) in combination with BAP (1.0 mgL^{-1}). At the 6th week of culture, early development of globular embryos with meristemic centre surrounded by cells with vacuoles was observed (Figure 2A). These globular embryos were then separated from callus tissues and became single embryos (Figure 2B) and further develop into heart-shaped stages (Figure 2C).

4. DISCUSSION

The objective of this project was to develop a method for induction of somatic embryogenesis of *B. pavonina* using *in vitro* leaf explants. To our knowledge, this is the first report of somatic embryogenesis *B. pavonina*. Explant cultured on MS medium supplemented with 2,4-D (1.0 and 2.0 mgL^{-1}) in combination with BAP (1.0 mgL^{-1}) has successfully induced callus within four weeks in culture, and further developed into somatic embryos. MS containing lower concentration of 2,4-D (0.5 mgL^{-1}) and 1.0 mgL^{-1} BAP or 2,4-D alone induce shoot organogenesis or were unable to stimulate an embryogenic response. Similarly, leaf and petiole explants of *Dioscorea zingiberensis* did not form embryogenic cultures on MS media with various concentrations of 2,4-D as the sole growth regulator [24]. Shoot initiation from callus of Sentang was reported in cultures supplemented with lower auxin and BAP in induction medium [25].

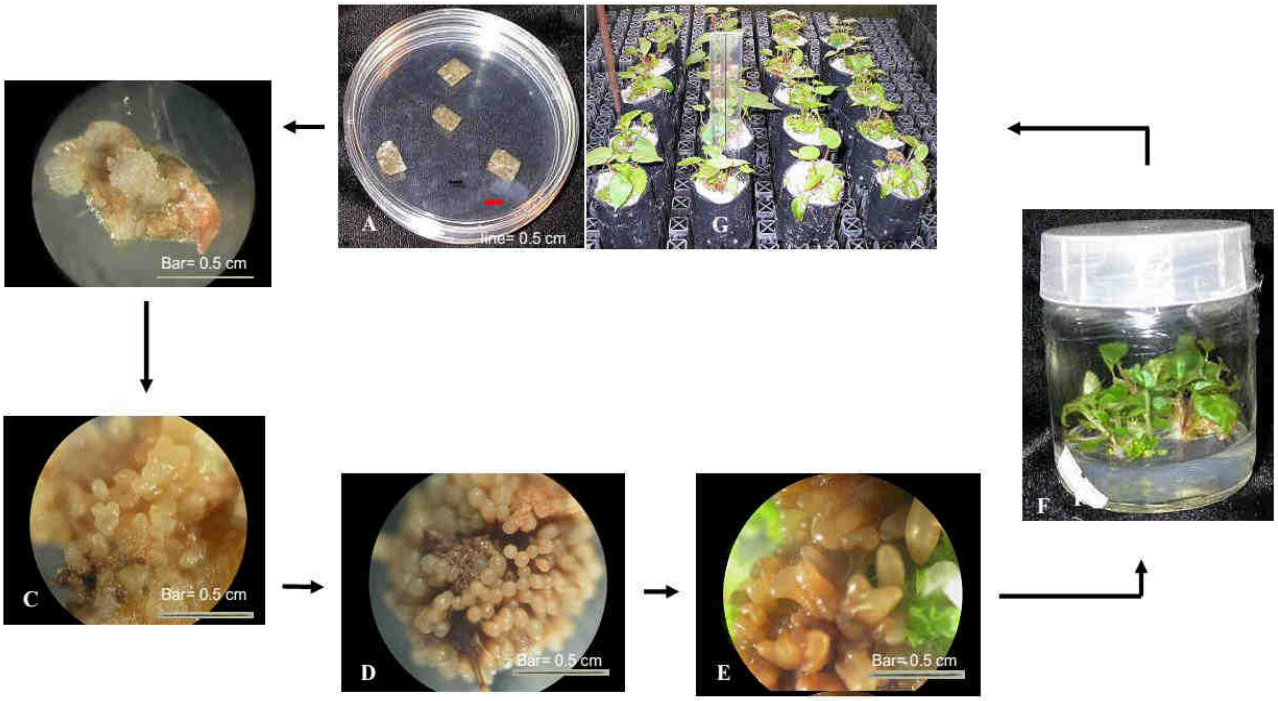

Figure 1. Developmental pathways of somatic embryogenesis of *B. pavonina*: (A) leaf explants cultured on callus induction medium, (B) callus initiation after two weeks in culture, (C) callus covered the whole explants after four weeks, (D) globular embryos attached to callus tissues, (E) torpedo and heart shaped structure, (F) plantlets derived from embryos and finally, (G) transplanted plants in potting medium.

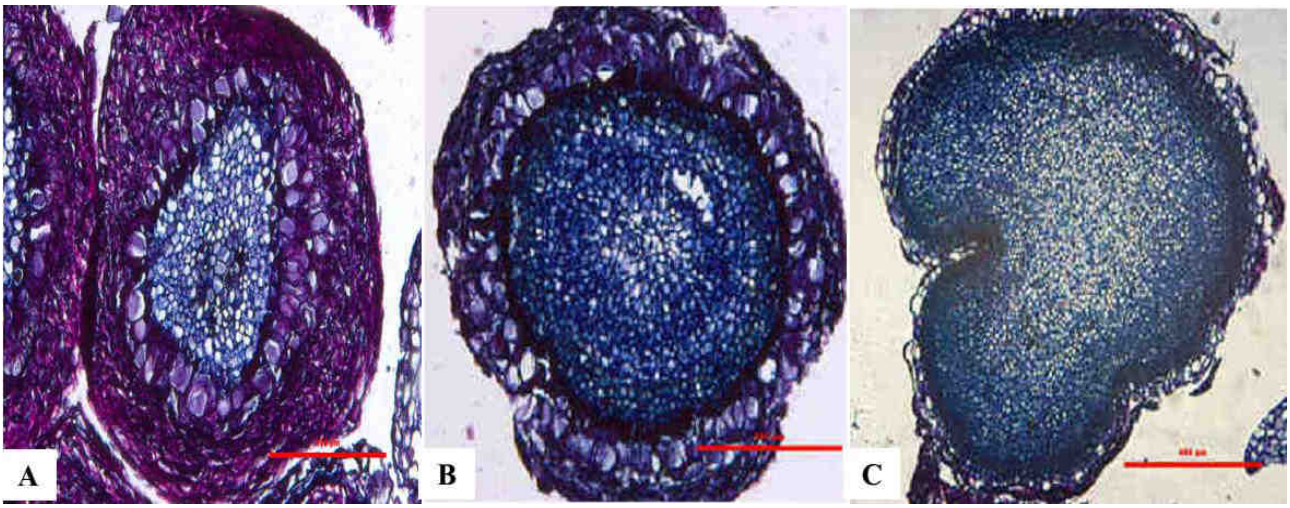

Figure 2: Histological examination of somatic embryos of *B. pavonina*. (A) Early development of globular embryos, scale bar 100 µm, (B) Globular embryos were separated from the callus tissues and became single embryos, scale bar 100 µm, (C) Heart embryo-like structure, scale bar 400 µm.

However, some multiple embryos were also produced, which were generally fused to each other and were difficult to separate into individual embryos. It is difficult to calculate the number of somatic embryos because some of them were clustered. Some somatic embryos were loosely attached to explants and were easily detached individually. Nevertheless, this study has demonstrated successful development of complete plantlets from somatic embryos.

Somatic embryogenesis is an important practice for conserving overexploited and endangered species. Since *B. pavonina* is known to be found only in the Cameron Highlands (Peninsular Malaysia), large scale propagation of this valuable Begonia is vital. Direct somatic embryogenesis has been reported for a number of Begonia species [3, 22, 26]. The data reported here demonstrated for the first time the plantlet regeneration from somatic embryos of *B. pavonina* from *in vitro* leaf explants. This approach can be a model study in propagating and conserving other endemic species of Begonia in Malaysia for example *Begonia rajah* (Johor), *Begonia abdullahpieei* (Perak), *Begonia sibthorpiodes* (Kedah), *Begonia phoeniogramma* (Selangor), *Begonia isopteroidea* (Pahang), *Begonia jiewhoei* (Kelantan), *Begonia alpine* (Pahang), *Begonia koksunii* (Perak) and *Begonia tigrina* (Kelantan). In addition, the protocol demonstrated here can also be used for regeneration of Begonia species through direct somatic embryogenesis for various purposed including ex-situ conservation, regeneration and for raising plant material for genetic manipulation.

AUTHORS' CONTRIBUTION

Conception and design: AAR; Development of methodology: AAR, KIK, PN, QZF; Acquisition of data: AAR; Analysis and interpretation of data, writing, review and/or revision of the manuscript, administrative, technical or material support: AAR, KIK, PN; Study supervision: KIK. All authors are involved in drafting the manuscript, read and approved the final manuscript.

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TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Fusarium species and other fungi associated with some seeds and grains in Egypt, with 2 newly recorded *Fusarium* species

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ABSTRACT

Seventeen species of *Fusarium* were recorded on maize (6 species) and sorghum (12 species) grains, and lentil (7 species) and sesame (7 species) seeds on Dichloran chloramphenicol peptone agar medium (DCPA) using the seed/grain-plate method. The moisture contents of most cereal grains were higher than those of seeds. Maize grains gave the highest colony forming units of *Fusarium* followed by sorghum, sesame and lentil. The frequency of occurrence of *Fusarium* species depended on the type of seeds and grains. The most frequently encountered species were *F. oxysporum, F. verticillioides, F. solani* and *F. proliferatum* on maize; *F. nygamai, F. solani* and *F. verticillioides* on sorghum, *F. nygamai* and *F. oxysporum* on lentil; and *F. solani, F. chlamydosporum* and *F. verticillioides* on sesame. *Aspergillus* (with *A. flavus* and *A. niger* being the most common) followed by *Penicillium* and *Alternaria* were recorded from the 4 substrates.

Key words: *Fusarium*; Cereal grains; Seeds; *Fusarium acutatum; Fusarium nisikadoi*.

INTRODUCTION

Fungi carried on or within grain or seed can reduce grain or seed germination or seedling emergence [1]. Some plant pathogenic fungi kill seedlings shortly after they emerge, whereas others cause serious disease epidemics after being transmitted from grain/seed to seedlings. Determining what proportion (incidence) of seeds in a given seed lot are contaminated by a fungus (either externally or internally) is therefore of interest to plant disease epidemiologists [2, 3]. Gilbert *et al*. [4] reported that use of the infected seed/ grain without treatment results in lower plant densities. The natural contamination of seeds with seed-borne fungi plays a vital role in determination of seed quality [5].

Maize is one of the most important dietary staple foods in the world especially African people [6, 7]. In Egypt, maize is one of the most important and essential crops, especially in Upper Egypt, not only as food for animal and human but also for Egyptian economics because the crop is used mainly in several food industries [8]. Several fungi are associated with maize during pre- and post-harvest periods, of which the genus *Fusarium* contains important toxigenic species [9]. These include *F. verticillioides* which is one of the most economically important species worldwide [10-13]. Many studies to evaluate the natural occurrence of *Fusarium* in maize have been conducted in several parts of the world [10, 12]. Kossou and Aho [14] reported that fungi could cause about 50-80% of damage on farmers' maize during the storage period if conditions are favorable for development.

Sorghum is the fourth most important cereal in Egypt (after maize, wheat and rice), and is the only one of these cereals that can be easily cultivated in the "new lands' or in very hot and arid Upper Egypt [15]. *Fusarium* species in the *Gibberella fujikuroi* species complex are widely known from maize and sorghum in Egypt. A common perception is that cause stalk; ear and kernel rot and produce mycotoxins such as fumonisins and moniliformin [16].

Thirteen species of *Fusarium* were reported earlier, but with different counts and incidences from some Egyptian cereals grains [17-25]. Aziz *et al*. [26] found that *Fusarium* infection of wheat, maize and barley grains ranged from 25% to 40%, 30% to 60% and 10% to 25%, respectively. Five species of *Fusarium* were collected and the most common species was *F. moniliforme* (38.6% of total *Fusarium)* followed by *F. proliferatum* (29%), *F. graminearum* (16.5%), *F. subglutinans* (9.1%), and *F. oxysporum* (6.8%).

Lentil seed is one of the oldest known protein-rich food legumes [27]. Lentil wilt, caused by *Fusarium oxysporum* f. sp. *lentis* is one of the main limiting factors to successful cultivation [28]. Sesame seed is also an important oilseed widely grown and used in some African and Asiatic countries. It is an important source of protein in the developing countries and the name Benniseed is used throughout West Africa [29].

Sesame oil is mainly utilized as a salad and cooking oil or in the manufacturing of margarine. Abd-Allah and Hashem [30] could isolate 32 species belonging to 17 genera from lentil seeds, of which *Fusarium* species (*F. moni-liforme, F. solani, F. semitectum, F. equiseti, F. oxysporum* and *F. roseum*) were of high frequency of occurrence. Embaby and Abdel--Galil [31] found that *Fusarium* was the common genus isolated from some legume seeds (bean, cowpea and lupine), emerging in 5.6%, 4.4% and 4.4% of total fungi, respectively of which *F. oxysporum* was the most common species.

The aim of this investigation was to study the composition and distribution of fungi especially *Fusarium* of sorghum and maize grains and lentil and sesame seeds.

2. MATERIALS AND METHODS

2.1. Collection of samples

Forty grain and seed samples were collected from different markets at Assiut city (10 samples of each of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), lentil (*Lens culinaris* Medic.), and sesame (*Sesamum indicum* L.). The samples were put in clean polyethylene plastic bags and brought to the mycological laboratory till fungal analysis.

2.2. Determination of moisture content of samples

The moisture content was estimated by drying 50 g of the grains or seeds which were ground in a blender and put in an oven for 24 h at 105°C, then cooled in a desiccator and re-weighed. The moisture content is expressed as percentage of the dry-weight.

2.3. Isolation and identification of fungi

The direct plating technique adopted by the second international workshop [32] was used to isolate and enumerate fungi in grain and seed samples. Twenty five grains or seeds from each sample were put on the surface of the isolation medium (5 grains or seeds per each of 5 plates for each sample). The plates were incubated at 25°C for 7 days and the developing fungi were counted, isolated, identified and calculated as colony forming units (CFU) per 25 grains or seeds in each sample.

The isolation medium used was dichloran chloramphenicol peptone agar (DCPA). This medium was developed for selective isolation of *Fusarium* species and dematiaceous hyphomycetes from cereals [33]. It contains per liter distilled water: peptone, 15 g; KH_2PO_4, 1 g; $MgSO_4$. $7H_2O$, 0.5 g; dichloran (0.2% solution in ethanol), 1 ml; chloramphenicol, 0.2 mg; agar, 15 g. The medium was sterilized at 121°C for 15 min; the final pH was 6.2.

Identification of *Fusarium* species was carried out following the procedures of Leslie and Summerell [16], Booth [34], Nelson *et al*. [35], Nirenberg *et al*. [36]. Potato sucrose agar (PSA) and potato dextrose agar (PDA) were used for identification.

3. RESULTS AND DISCUSSION

The moisture contents of most cereal grains were higher than those of seeds, where they ranged from 8.75-16.76% in maize and 7.16-13.63% in sorghum; and 3.07-12.05% in sesame and 6.48-13.76% in lentil (Table 1). Abd-Allah and Hashem [30] noticed that, the moisture contents of the 45 lentil seed samples collected from the different governorates ranged between 5% and 13.15%.

The results of the present study (Table 2) showed that a collectively of seventeen species of *Fusarium* were isolated from maize (6 species) and sorghum (12) grains, and lentil (7) and sesame (7) seeds on DCPA medium using the seed/grain-plate method. The highest count was recorded on maize (148 CFU/25 maize grains) followed by sorghum (108), sesame (31) and lentil (26). The frequency of occurrence of *Fusarium* species was depended on the type of grains and seeds.

Fusarium was isolated from all samples of maize and sorghum grains investigated; constituting 32.38% and 22.88% of total fungi respectively. Its count fluctuated between 6-24 and 1-26 CFU/25 maize and sorghum grains and the highest counts were obtained in maize sample No. 3 and sorghum sample No. 1 which contained relatively high moisture content (9.35% and 7.16%). *Fusarium* was isolated from 6 and 9 samples of lentil and sesame seeds (out of 10 samples), comprising 13.98% and 12.97% of total fungi respectively. Its count fluctuated between 2-6 and 1-13 CFU/25 seeds and the highest count was obtained in lentil samples Nos. 1 & 2 which contained 6.48% and 11.52% moisture contents and in sesame sample No. 1 which contained 5.76% moisture content.

The most frequently encountered species were *F. oxysporum*, *F. verticillioides*, *F. solani* and *F. proliferatum* on maize, comprising 18.92%, 31.08%, 15.54% and 25% of total *Fusarium* and 6.13%, 10.07%, 5.03% and 8.09% of total fungi, respectively. *F. udum* was isolated in low frequency, while *F. nisikadoi* was of rare occurrence (Table 2). On sorghum, *F. nygamai* and *F. verticillioides* were isolated in high frequency of occurrence and *F. solani* in moderate frequency, comprising 20.37%, 25% and 13.89% of total *Fusarium* and 4.66%, 5.72% and 3.18% of total fungi, respectively. *F. chlamydosporum* and *F. oxysporum* were isolated in low frequency from sorghum, while the other 7 *Fusarium* species were rarely isolated and these include *F. anthophilum, F. nisikadoi, F. pseudoanthophilum, F. pseudonygamai, F. semitectum, F. thapsinum* and *F. udum* (Table 2). It is worthed to mention that *F. nisikadoi* was isolated here for the first time in Egypt from cereal grains.

F. nygamai and *F. oxysporum* were isolated in low frequency of occurrence from lentil seeds. They emerged each in 40% of the samples, comprising 23.08% and 19.23% of total *Fusarium* and 3.23% and 2.69% of total fungi, respectively. *F. solani* was isolated in moderate frequency from sesame seeds while *F. chlamydosporum, F. verticillioides* and *F. subglutinans* were isolated in low frequency of occurrence, comprising 35.48%, 22.58%, 12.9% and 16.13% of total *Fusarium* and 4.6%, 2.93%, 1.67% and 2.06% of total fungi, respectively. More 5 *Fusarium* species were rarely found on lentil and these were *F. anthophilum, F. subglutinans, F. udum, F. verticillioides* and *F. xylarioides* (Table 2) and 3 species from sesame and these were *F. acutatum, F. graminearum* and *F. xylarioides* (Table 2). Of these *F. acutatum* is being recorded here as a new record in Egypt.

Most of the above species were previously isolated from various types of seeds and grains in Egypt [17-25]. Thirty-two species belonging to 17 genera were recovered from lentil seeds, of which *Fusarium* species (*F. moniliforme, F. solani, F. semitectum, F. equiseti, F. oxysporum* and *F. roseum*) were isolated in high frequency of occurrence [30]. Embaby and Abdel-Galil [31] found that *Fusarium* was the common species isolated from some legume (bean, cowpea and lupine), emerging in 5.6%, 4.4% and 4.4% of total fungi, respectively and the most common species was *F. oxysporum*. Aziz *et al.* [26] found that *Fusarium* infection of wheat, maize and barley grains ranged from 25% to 40%, 30% to 60% and 10% to 25%, respectively. Five species of *Fusarium* were collected and the most common species was *F. moniliforme* (38.6% of total *Fusarium)* followed by *F. proliferatum* (29%), *F. graminearum* (16.5%), *F. subglutinans* (9.1%), and *F. oxysporum* (6.8%).

Table 1. Moisture content (MC %), colony forming units of all fungi (TCFU), colony forming units of *Fusarium* (FCFU) and number of fungi-free grains (NFG) or seeds (NFS) recorded from 10 samples of each of maize and sorghum grains, and lentil and sesame seeds (25 grains or seeds each sample) on Dichloran chloramphenicol peptone agar DCPA at 25ºC.

Grain/Seed	Maize				Sorghum			
Sample No	MC	TCFU	FCFU	NFG	MC	TCFU	FCFU	NFG
1	13.25	65	16	0	7.16	61	26	0
2	10.56	38	11	0	9.76	52	2	0
3	9.35	69	24	0	11.95	52	19	0
4	17.3	59	19	0	10.25	49	6	0
5	12.56	57	20	0	8.44	52	13	0
6	15.21	43	22	0	13.63	42	7	0
7	8.75	41	11	0	11.4	42	9	0
8	9.62	44	12	0	8.9	35	11	0
9	16.76	30	6	5	12.02	46	14	0
10	14.87	11	7	16	9.38	41	1	0
Mean± SD	12.8±3.16	45.6±17.53		2.1±5.13	10.29±1.96	47.2±7.49		0

Grain/Seed	Lentil				Sesame			
Sample No	MC	TCFU	FCFU	NFS	MC	TCFU	FCFU	NFS
1	6.48	20	6	8	5.76	45	13	0
2	11.52	21	6	7	10.71	3	1	22
3	10.6	16	0	11	11.42	25	4	2
4	8.2	17	0	8	12.05	7	1	18
5	9.65	15	5	6	3.07	31	4	0
6	13.76	27	2	1	6.72	16	4	12
7	9.23	21	4	6	9.53	28	2	0
8	7	13	0	11	4.11	25	0	5
9	9.03	23	3	3	6.48	34	1	0
10	6.6	12	0	12	9.6	25	1	5
Mean± SD	9.21±2.32	18.5±4.72		7.3±3.53	8.38±3.17	23.9±12.49		6.4±8.14

Other 12 fungal genera were recorded also on the used medium from maize (7), sorghum (6), lentil (4) and sesame (8) of which *Aspergillus* was isolated in high frequency from all samples of all substrates with *A. flavus* and *A. niger* are the most frequent. *Penicillium* was also isolated from all substrates but more frequent in lentil and sesame seeds. *Alternaria* was also isolated from all substrates but in low frequency from sorghum and lentil and in rare frequency from maize and sesame (Table 2). The remaining species were recorded mostly in rare frequency on one substrate or more (Table 2). Also, it is worthed to mention that none of the sorghum grains tested was fungi-free, while 21 of the 250 maize grains tested were fungi-free. On the other hand, large numbers compared to that in maize grains (64 sesame and 73 lentil seeds) were fungi-free.

3.1. Brief descriptions of the newly recorded species

3.1.1. *Fusarium acutatum* Nirenberg & O'Donnell 1998

Colonies on PSA showing growth rate varies from 4.45-5.25 cm diameter, with a mean ± SD = 4.9±0.41, aerial mycelium white and pigmentation light orange (M. 5A4) according to the index colour of Kornerup and Wanscher [37]. Macroconidia present and sparse, falcate, thin walled, usually 3-septate, 30-54 x 2-3.5 µm with apical cell bent and foot-shaped basal cell. Microconidia abundant,

Table 2. Colony forming units (CFU) and number of cases of isolation (NCI) and occurrence remarks (OR) of *Fusarium* species and other fungi isolated from 10 samples of each of maize and sorghum grains, and lentil and sesame seeds on Dichloran chloramphenicol peptone agar (DCPA) at 25 °C.

Grain/seed	**Maize**		**Sorghum**		**Lentil**		**Sesame**	
Taxa	CFU	NCI&OR	CFU	NCI&OR	CFU	NCI&OR	CFU	NCI&OR
***Fusarium* (total)**	148	10 H	108	10 H	26	6 M	31	9 H
F. acutatum Nirenberg & O' Donnell	0	0	0	0	0	0	1	1 R
F. anthophilum (A. Braun) Wollenw.	0	0	2	1 R	2	1 R	0	0
F. chlamydosporum Wollenw. & Reinking	0	0	10	3 L	0	0	7	4 L
F. graminearum Schwabe	0	0	0	0	0	0	1	1 R
F. nygamai L. W. Burgess & Trimboli	0	0	22	7 H	6	4 L	0	0
F. nisikadoi T. Aoki & Nirenberg	2	1 R	2	1 R	0	0	0	0
F. oxysporum Schltdl.	28	7 H	11	4 L	5	4 L	0	0
F. proliferatum (Matsush.) Nirenberg	23	5 M	0	0	0	0	0	0
F. pseudoanthophilum Nirenberg, O' Donnell & Mubatanhema	0	0	3	1 R	0	0	0	0
F. pseudonygamai O' Donnell & Nirenberg	0	0	3	1 R	0	0	0	0
F. semitectum Berk. & Ravenel	0	0	4	2 R	0	0	0	0
F. solani (Mart.) Sacc.	37	5 M	15	5 M	0	0	11	5 M
F. subglutinans (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas	0	0	0	0	3	2 R	4	3 L
F. thapsinum Klittich, Leslie, Nelson & Marasas	0	0	2	2 R	0	0	0	0
F. udum E. J. Butler	12	3 L	7	2 R	3	2 R	0	0
F. verticillioides (Sacc.) Nirenberg	46	7 H	27	7 H	3	2 R	5	4 L
F. xylarioides Steyaert	0	0	0	0	4	2 R	2	2 R
Other fungi (total)	309	10 H	364	10 H	160	10 H	208	10 H
Alternaria spp.	4	1 R	11	4 L	8	4 L	6	2 R
Aspergillus (total)	257	10 H	324	10 H	101	10 H	177	10 H
A. flavus Link	122	10 H	187	10 H	25	9 H	61	8 H
A. niger van Tieghem	79	9 H	136	10 H	73	10 H	109	10 H
A. ochraceus Wilhelm	0	0	0	0	2	2 R	6	2 R
A. tamarii Kita	7	2 R	1	1 R	0	0	0	0
A. terreus Thom	0	0	0	0	0	0	1	1 R
Aspergillus spp.	49	8 H	0	0	1	0	0	0
Botryotrichum spp.	4	1 R	0	0	0	0	0	0
Cladosporium spp.	4	1 R	0	0	6	5 M	8	3 L
Eurotium sp.	0	0	0	0	0	0	1	1 R
Gliocladium sp.	0	0	0	0	0	0	1	1 R
Humicola sp.	0	0	4	1 R	0	0	0	0
Penicillium spp.	28	3 L	18	4 L	45	10 H	13	5 M
Rhizopus sp.	0	0	0	0	0	0	1	1 R
Setosphaeria spp.	8	2 R	6	2 R	0	0	1	1 R
Trichoderma sp.	0	0	1	1 R	0	0	0	0
Trichothecium roseum (Persoon: Fries) Link	4	1 R	0	0	0	0	0	0
Gross total	**457**	**10**	**472**	**10**	**186**	**10**	**239**	**10**
No. of genera	8		7		5		9	

oval, fusoid, 0-septate, 5-10 x 1.5-2.5 µm, found only in false heads from mono- and polyphialidic conidiogenous cells. Chlamydospores formed slowly, in chains and in clusters. *F. acutatum* may be confused with the closely related *F. anthophilum* and *F. subglutinans* since all produce microconidia in false heads on mono- and polyphialidic conidiogenous cells. *F. acutatum* could be distinguished from both by its orange pigmentation and macroconidia with acute apical cells. Also, *F. acutatum* could be distinguished from *F. anthophilum* morphologically by their microconidial shape. This species was first described by Nirenberg and O'Donnell [38] for those cultures forming acute or bent macroconidia. Leslie and Summerell [16] stated that this species needs to be confirmed and validated with a large number of isolates from diverse sources. Isolates of *F. acutatum* produce trace levels of beauvericin and fumonisins [16, 39].

3.1.2. *Fusarium nisikadoi* T. Aoki & Nirenberg 1997

Growth rate on PSA after 4 days ranges from 5.45-5.9 cm diameter, with a mean ± SD = 5.65 ± 0.35, aerial mycelium white with orange pigmentation (M. 6A6) [37]. Macroconidia long, straight to falcate, 3-7 septate, 32-70 x 3.5-5 µm, with acute apical cell and foot shaped basal cell, produced 2-3 weeks of incubation. Microconidia abundantly clavate 0-3 septate, 6-22 x 2.5-4 µm, but occasionally pyriform 0-septate, 7.5-11.5 x 5-9.5 µm. Microconidia are produced in long chains and false heads on mono- and polyphialidic conidiogenous cells, chlamydospores absent. *F. nisikadoi* can be distinguished from *F. verticillioides*, *F. thapsinum* and *F. proliferatum* by the presence of microconidia up to 3-septate. *F. nisikadoi* was isolated for the first time from *Phyyostachys nigra* var. *henonis* (bamboo) and *Triticum sativum* (wheat) in Japan by Nirenberg and Aoki [40]. Isolates of *F. nisikadoi* produce trace amounts of moniliformin [39].

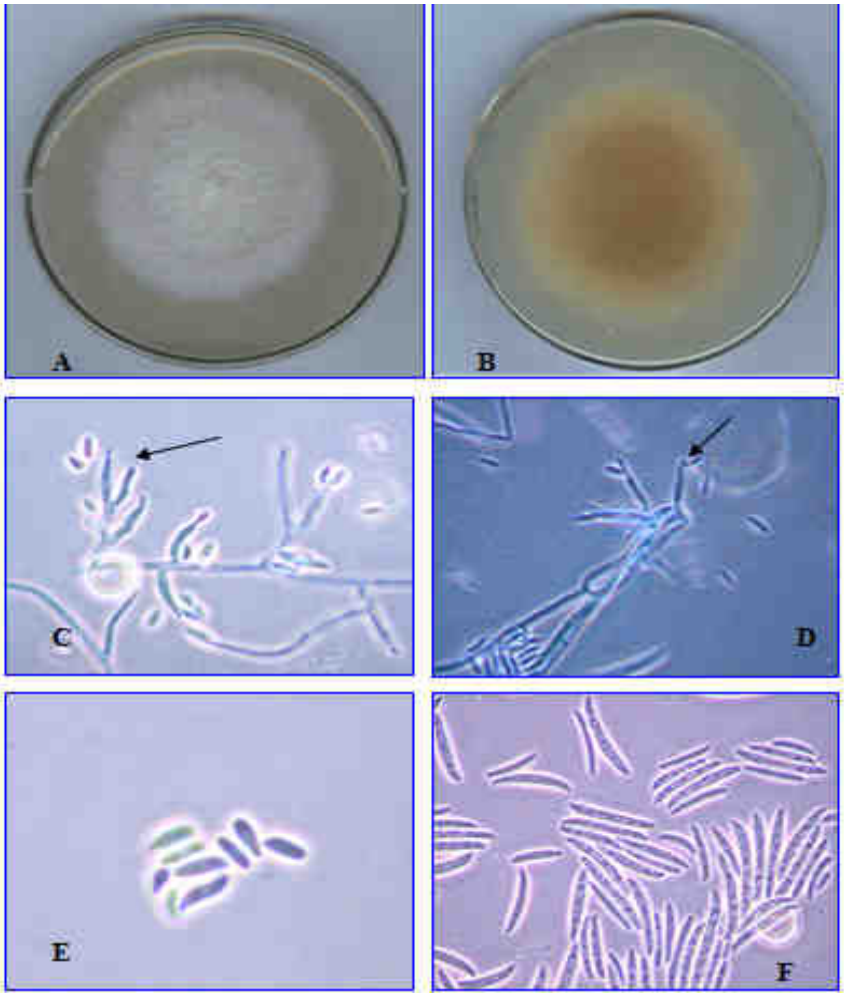

Plate 1. *Fusarium acutatum* Nirenberg & O'Donnell: A, B: Colony colour and reverse on PSA; A-F: Photographs; C-D: Mono- and polyphialidic conidiogenous cells; E: Microconidia; F: Macroconidia.

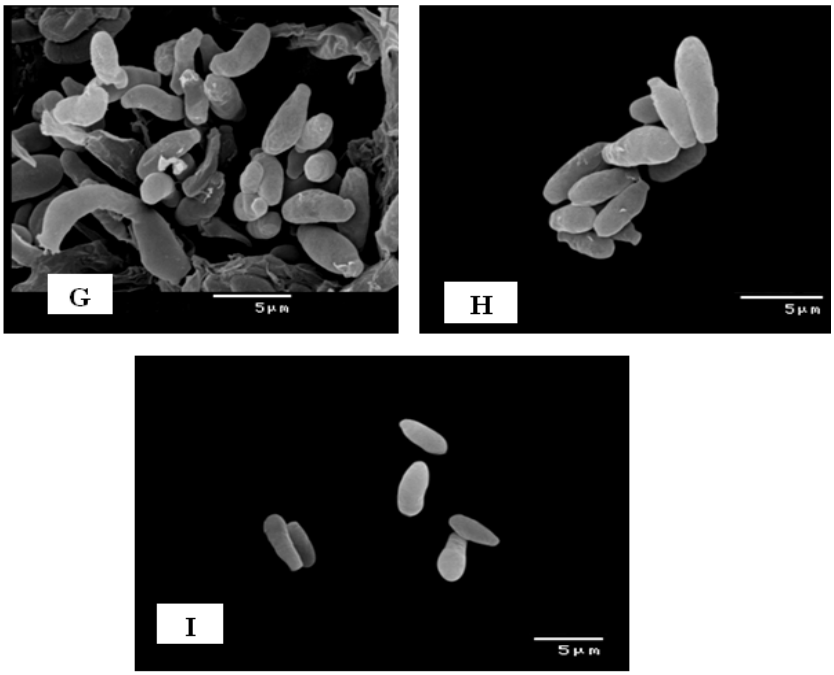

Plate 2. *Fusarium acutatum* Nirenberg & O'Donnell: G-I: Microconidia (Scanning electron micrographs).

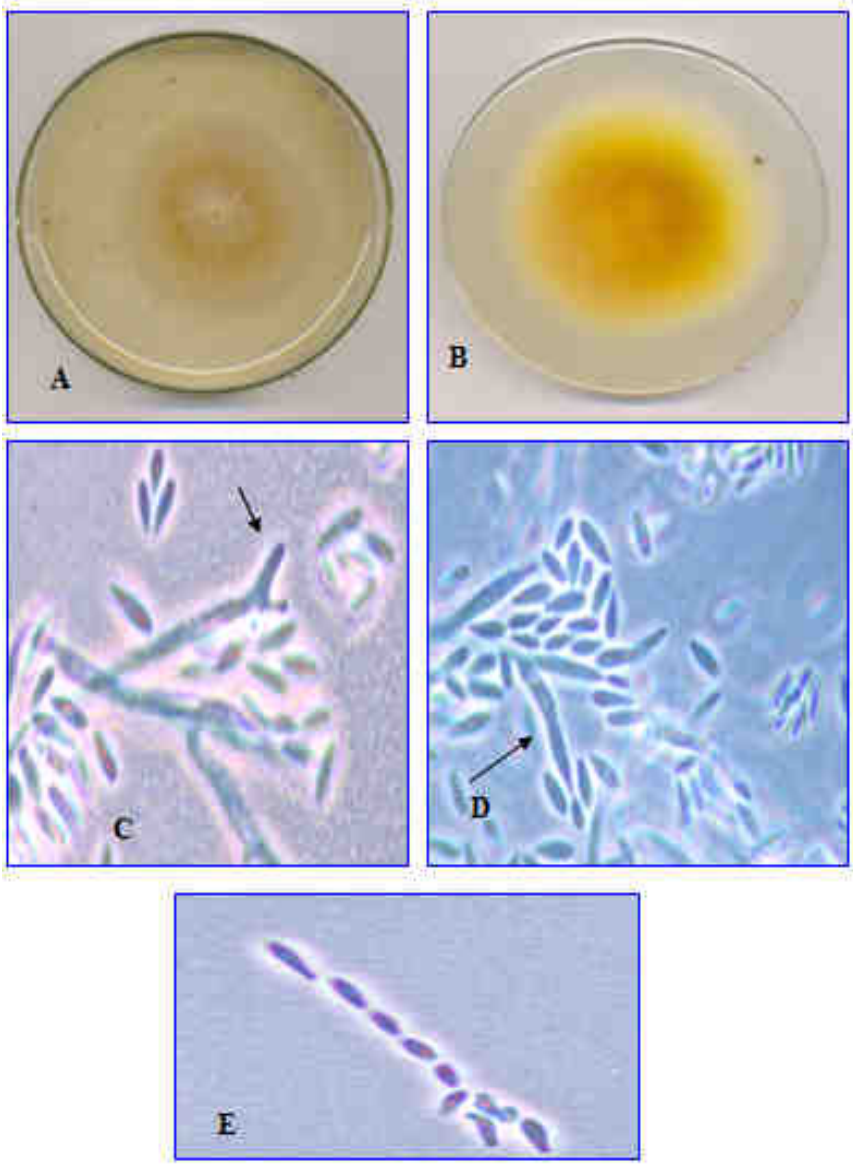

Plate 3. *Fusarium nisikadoi* T. Aoki & Nirenberg: A, B: Colony colour and reverse on PSA; A-E: Photographs: C, D: Poly- and monophialidic conidiogenous cells; E: Microconidia in chains.

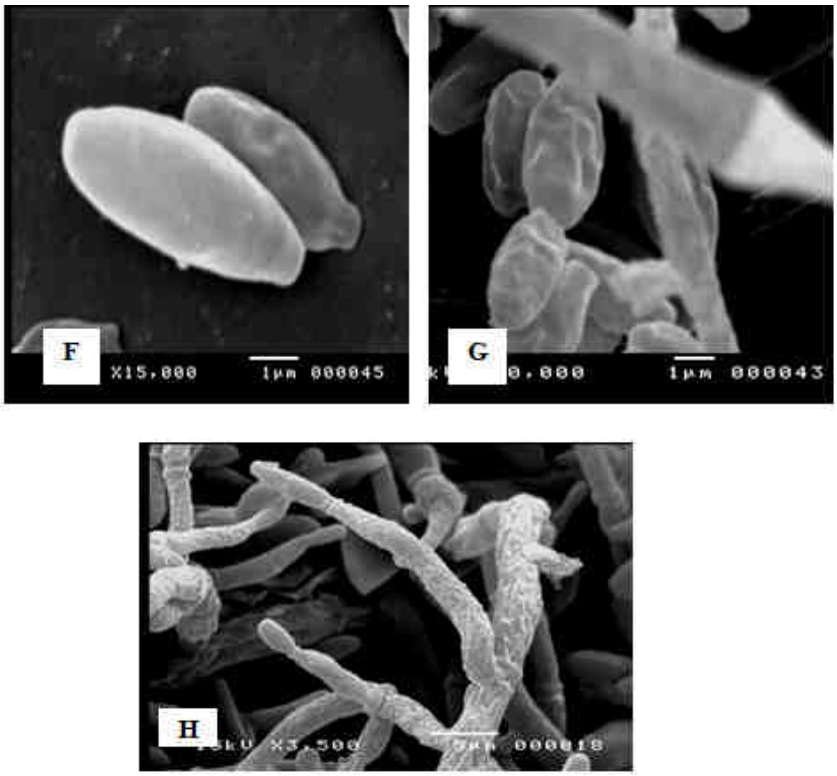

Plate 4. *Fusarium nisikadoi* T. Aoki & Nirenberg, F-H: SEM; F, H: Micro-conidia; H: Monophialidic conidio-genous cells.

AUTHORS' CONTRIBUTION

Study design, Data collection, Statistical analysis, Data interpretation, Manuscript preparation, Litera-ture search, and Funds collection: SIIA-H, MAI, NAH and NAA-H. All authors are involved in drafting the manuscript, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Bamboo: potential resource for eco-restoration of degraded lands

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ABSTRACT

Bamboo forests are important forest type in subtropical and tropical region in the world. Due to its biological characteristic and growth habits, bamboos are not only an ideal economic investment that can be utilized in many different manners but also has enormous potential for eco-restoration of degraded lands. Bamboos are one of those communities which rapidly colonized disturbed lands due to their adaptability and nutrient conservation ability. Bamboo protects steep slopes, soils, water ways, prevents soil erosion, sequester carbon and brings many other ecosystem benefits. The impact of bamboo growth on the soil may be different at their species level and it is expected that there is a large increase in the microbial biomass, particularly, in the rhizosphere zone as they do not provide only a larger root surface area but enhances the soil fertility. The important role of microbial biomass in enhancement of soil fertility has been evaluated in various terrestrial ecosystems and found to play crucial role in nitrogen and phosphorous dynamics. Hence, data pertaining to the microbial biomass influenced by bamboo growth need to be used as a potential index for soil nutrient recovery during the restoration of degraded ecosystems. The role of bamboo in eco-restoration of degraded land has received huge attention of ecologists, foresters and soil scientists. However, further extensive research is required for better insights in this aspect.

Key words: Degraded land; Bamboo; Eco-restoration; Nutrient dynamics.

1. INTRODUCTION

Bamboos are one of the most versatile and widely utilized flowering perennials of Poaceae family. These are the biggest members of the grass family, having hollow inter nodal regions with scattered vascular bundles throughout the stem in a cylindrical arrangement. There are nearly 1500 species under 87 genera of bamboos worldwide [1]. Bamboos are one of the most important species particularly in Asia, where it is frequently considered as the “timber of the poor” [2]. It has been used for many applications, from a food source to construction materials. Further, it is a potential source of essential oils and medicines [3]. Bamboo, one of the fastest-growing plants on earth due to its unique rhizome dependent system [4], with reported growth rates of 250 cm in 24 hours. However, the growth rate is dependent on local soil and climatic conditions, as well as on the species. Bamboo can be used in many ways according to the different problems, no matter what they are socially, environmentally or economically. Bambusetum of Rain Forest Research Institute (Jorhat, India) is presented on Fig. 1, and Bamboo Plantation in degraded Jhoom lands of Shercip, on Fig. 2.

Figure 1. Bambusetum of Rain Forest Research Institute, Jorhat, Assam, India.

Figure 2. Bamboo Plantation in degraded Jhoom lands of Shercip, Mizoram.

Bamboo forests have ecological and environmental functions in term of soil erosion control, land rehabilitation, water conservation and carbon sequestration [5]. The rapid increase in the rate of deforestation makes bamboo an ideal investment or choice for plantation. Biological characteristics and growth habits of bamboo make it more important in solving the problem of degraded lands, like for erosion control [6] and carbon sequestration. Bamboos in the future may be able to increase the bio-capacity by simultaneously increasing the area of fertile global hectares that is able to supply resources [7]. Land degradation has raised one of the serious debates, as it is become an important issue in the modern era due to decrease in the agriculture and forest area.

Land degradation is defined as the long-term loss of ecosystem function and productivity caused by disturbances from which the land cannot recover unaided [8]. It is the net result of derivative processes regulated by natural and anthropogenic factors. The degree of soil degradation depends on soil's susceptibility to degradative processes, land use, duration of degradative land use and the management processes. Hence, due to the decrease in the productive area, encroachment of humans in forest and agricultural lands to ensure food security in future, ecological restoration of degraded lands has paramount importance.

Ecological restoration is defined as the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed or it is the return of a damaged ecological system to a stable, healthy, and sustainable state. The basic

concept behind restoring any degraded land includes: having exact knowledge and monitoring about the actual condition of the problem, including the causes and their impact and this also includes measures to conserve nutrients in degraded lands. There are so many approaches for the eco-restoration of degraded lands like mechanical and biological approach, proper land use planning, restoration of mined areas, soil amendments for enhancing productivity, improvement of shifting agriculture, afforestation and agroforestry.

Mechanical approaches are used in highly degraded areas, where other approaches are either works slow or not possible, but these are much expensive and require maintenance. Biological approaches are economically sound for restoration of degraded lands due to better soil protection capacity and most effective when amalgamated with mechanical approaches. Proper land use planning, aimed for optimization of production from degraded land as well as from conservation point of view. Ecological rehabilitation of mined areas requires capital and can be attempted by using plant species of economic value to local population. Another way to restore degraded land is soil amendments, as they improve physical, chemical and biological properties of the soil. Improvement of shifting cultivation areas is another way of eco-restoration and it can be archived by introduction of plantation crop in Jhum fields [9]. Afforestation and agroforestry, is most important approach from hydrological and erosion control point of view, but the most important thing is that all the necessities of farmers, fuel, food and timber are supplemented from the single patch of land. As, biological approach is an effective and most economical, so there is a dire need to introduce fast growing bamboo species in restoration of degraded lands. In this article an attempt has been made to discuss the importance of bamboos in eco-restoration of degraded lands.

2. BAMBOO IN ECO-RESTORATION

Bamboos are known to grow in "poor soils" and therefore used for rehabilitation of degraded lands [10]. It can be grown under diverse environmental conditions such as in full sunlight to the areas of high winds. This enables it to be used as a starting point in restoring degraded land. Adaptive capability, nutrient and water conservation of bamboos, enables it as fore-runner plants in the eco-restoration of degraded land. Because of the fast growing nature and the dense foliage of bamboos, it is able to maintain the thick layer of litter. This litter layer maintains microclimate in the understory and soil moisture, the most important factors for the restoration of degraded lands. Bamboo shoots and culms grow from the dense root rhizome system. There are mainly two types of rhizomes: monopodial and sympodial. Monopodial rhizomes grow horizontally, at a surprising rate, thus their nickname of ‘runners’ or ‘running bamboo’. The rhizome buds develop either upward, generating a culm, or horizontally, with a new tract of the rhizome net. Bamboos grown on the hill slopes of degraded jhum land, will be able to control the runoff and soil erosion because of the presence of intensive root system. There are very few reports describing the rapid colonization of bamboos in the degraded lands. Different species of bamboos affect soil properties in different ways; among them some have been reported to increase the microbial biomass in rhizosphere zone by providing the large root surface, which helps in increasing the soil fertility [11]. Venkatesh et al. also concluded from his study that out of 11 studied species, *D. gigantells*, *D. hookerii* and *B. nutans*, has been found to be the better species for improving and maintaining the fertility status of acid soils in the NEH region [12]. Role of microbial biomass in soil productivity is already well known as it plays a major part in dynamics of major nutrient like nitrogen and phosphorus [7]. It also acts as a ‘sink’ and ‘source’ of the available plant nutrients. Mixed bamboo stands exhibit elevated amounts of advantageous soil nutrients and superior soil qualities as compared to monoculture stands, including soil porosity, aeration and bulk density [13]. Introduction of bamboo enriches soil fertility and microbial activities and also soil enzyme activity [5]. Besides this, physiochemical properties of soil were significantly better in the bamboo forests [14].

Dry matter and nutrient return to soil through litterfall and root mortality are the major components of nutrient cycling in non-bamboo and bamboo forests around the world [15-17]. The

organic matter returns to soil undergo different stages of decomposition depending upon the nature of substrate and the climatic conditions [18, 19] to generate the soil nutrients which support plant growth and thus C sequestration. There were two stages of litter decomposition in most litter fractions, a quicker stage during the first year and a slower stage during the second year. Previous studies attributed the initial mass loss of litter to the leaching of soluble C initially present, and to a high microbial activity, based on the most easily degradable compounds [20, 21]. During later stages, the decomposition rate was mainly negatively influenced by slowly degradable compounds, such as lignin, phenols, and tannins [21].

Table 1. Comparison of carbon stock in bamboo and tree forest ecosystems (t C/ha) [12].

Parts / Forest	Arbor & Shrub	Litter	In soil	Total	Ref.
Moso bamboo in Lin'an (medium-intensity management)	34.2	0.66	71.48	106.34	[35 and 36]
Chinese Fir at 15th year	53.60	3.43	93.16	203.79	[27]
Moso bamboo in Yong'an (medium-intensity management)	61.3	3.01	197.36	261.67	[18]
Deciduous board leaved forest	47.75	5.85	208.90	262.50	[34]

Table 1 shows that well managed bamboo forests are likely to be a lower static carbon store as compared with other forest types (varying from 122 t C/ha to 263 t C/ha). The quantity of carbon sequester by any forest type can be extremely influenced by diverse factors like climatic and soil. However, it can be realized that bamboo will sequester prominent amount of carbon, if managed sustainably.

Non-legume (*Dendrocalamus strictus*) can also play a considerable role to restore mine spoil habitats, in the same manner that has been specially reported for leguminous species [28]. Hence, data pertaining to the microbial biomass influenced by bamboo growth need to be used as a potential index for soil nutrient recovery during the restoration of degraded ecosystems. Degradation of soil due to erosion is a major threat in any ecosystem, as it reduces the sustainability and productivity. Bamboo is also well known for controlling soil erosion, as it grows and establishes itself very well on sloppy terrains, hill slopes, embankments and gullies etc. This feature is attributed due to the extensive fibrous and inter connected root system [29]. The leafy mulch and the dense foliage of bamboo, protects soil against the beating and scorching actions of the raindrops. Also the year wise production of new culms from the rhizomes provides the opportunity of harvesting bamboo without affecting biomass and soil. Generally, roots and rhizomes of bamboo form a woven net in the rhizosphere which helps in holding the soil. In several studies, it has been reported that most of roots and rhizomes of bamboo are present in top layer of soil i.e. 0-30 cm, which made it most effective in controlling soil erosion. Planting bamboo on the sides of riverbanks and streams, helps in protecting riverbanks from the erosive action of rivers as these helps in binding the soil tightly and secondly they grows well due to ample supply of moisture. Bamboo is quite effective in conserving soil erosion in ravines [30]. Introducing bamboos in the coastal sand has been proved as an effective measure to increase tree species in coastal forest shelterbelts [31]. Bamboos were found to be suitable for increasing the overall soil fertility and preventing soil erosion [32]. A model demonstrating intercropping of Bamboo groves with folder grass was found to be most effective in controlling soil erosion. Tiwari et al. also conducted various studies and found the conservation effect of bamboo plantation on improved soil health over a period of time [33].

3. CONCLUSION

Eco-restoration of degraded lands requires basic ideas about the geographic and climatic conditions of the degraded sites. Further, technical information/review on the related studies is a prerequisite to design suitable experiments in near future. Development of implementation and monitoring program to evaluate the success of the restoration projects in order to achieve desired information is also essential. Besides identification and scheduling, tasks, use of standard methods, estimation of cost benefit ratio of the projects should also be taken in to consideration for successful implementation of such kind of studies. The role of bamboo in eco-restoration of degraded land has received huge attention of ecologists, foresters and soil scientists. It has been summarized that bamboo plantation have had an impact in changing behavior towards soil erosion control, biodiversity conservation and increasing capacities in restoration of degraded lands. There is a need to integrate local people in restoration programs by increasing awareness on bamboo importance in the same. However, further extensive research is required for better insights in this aspect.

AUTHORS' CONTRIBUTION

Study Design: GM, Data Collection: GM and KG, Analysis and Data Interpretation: SP and RK, Manuscript Preparation: GM and KG, Literature Search: GM and RK, Writing, Review and Revision: GM and NS. All authors are involved in drafting the manuscript, read and approved the final version of manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Genotoxic and haematological effect of commonly used fungicide on fish *Clarias batracus*

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ABSTRACT

Application of synthetic pesticides is one of the methods used to increase agriculture production. Pesticides in agricultural runoff affect all the aquatic organisms. The result from this study reveals that mancozeb had some hematological and genotoxic effect on *Clarias batracus*. Fishes were exposed to sublethal concentrations (80% of LC_{50} of 24h) of mancozeb in terms of micronucleus assay and haematology. Micronucleus assay was carried out after 24, 48, 72 and 96 h. Peripheral blood was collected to analyse micronuclei. The numbers of micronuclei at 48 h were maximum. For haematology fishes were exposed for one week. Blood was assayed for selected haematological parameters. It was determined that this pesticide caused a decrease in haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell (RBC) levels, and increase in white blood cell (WBC) level.

Key words: Mancozeb; *Claria batracus*; Haematology; Genotoxicity; Micronuclei.

INTRODUCTION

Chemical pesticides are well recognized as an economic approach to control pests, at the same time such chemicals are highly toxic to other species in the environment. These chemicals even when applied in restricted areas are washed and carried away by rains and floods to nearby aquatic system, thereby affecting aquatic biota, specially fish, which is important due to their nutritive value [1-3]. Synthetic pyrethroids such as azodrin, cypermethrin, deltamethrin, fenvalerate and mancozeb are used to protect many fruit, vegetable, nut and field crops against a wide spectrum of fungal diseases and rust in order to increase agricultural production. However, they have harmful effects on aquatic environments and organisms [4-6]. Mancozeb is not toxic to rats [7, 8] at given oral doses of LD_{50}. However, it is moderately to highly toxic to fish and aquatic organisms [9-11].

Hematological study is important in toxicological research because a hematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound. A thin epithelial membrane separates fish blood from the water and any unfavorable change in the water body is reflected in the blood [12]. The knowledge of hematological characteristics of the fish is important in deter-

mining its health status, toxicological and parasitological investigations as well as selecting brooders for breeding purposes. Genotoxicity is a property possessed by some substances that makes them harmful to the genetic information contained in organism. Genotoxicity results in mutation which may results in tumor or cancer.

Although, some studies have demonstrated the toxic effect of various pesticides on different animals as butachlor in *Salmonella* sp. [13], induction of micronuclei in cat fish erythrocytes [14], accumulation in body tissue in fresh water fish *Channa punctatus* [15]. Hematological changes in some fish exposed to various toxicants, i.e. in *Cyprinus carpio* exposed to cypermethrin and fenvalerate [16], in *Tilapia mossambica* exposed to cadmium chloride [17], in *Ctenopharyngodon idella* exposed to fenvalerate [18] and in *Heteropneustes fossilis* exposed to deltamethrin [19], in *Rainbow Trout* exposed to mancozeb [20], genotoxic effect in *cirrhinus mrigala* exposed to butachlore [21]. *Channa punctatus* exposed to carbosulphan [22]. But, there is paucity of genotoxic and haematological information available on *Clarias batrachus* it is generally considered to be one of the most important catfish species for aquaculture as well as for its economic value as food in almost all over India.

To address the issue of haematology and genotoxicity in terms of micronucleus assay of this pesticide on *C. batracus* and to study the magnitude and scope of these effects, present studies were undertaken.

2. MATERIALS AND METHODS

2.1. Experimental herbicide

In experiments was used Mancozeb, trade name Dithane M-45, a widely used fungicide.

2.2. Experimental animal

Freshwater catfish *C. batracus* (35 ± 5 g in weight and 16 ± 2 cm in length) were collected from local outlets of Gorakhpur Distict. The collected fishes were maintained in glass aquaria containing 100 l de-chlorinated tap water for acclimatization to laboratory conditions for 1 week. The water in aquaria was aerated continuously and change everyday. The fishes were fed daily on commercial fish food. Fish were subjected to a prophylactic treatment by bathing twice in 0.01% $KMnO_4$ for 30 min at intervals of 24 h. The dead animals were removed from the aquaria to avoid any contamination. The physio-chemical properties of experimental water are: temperature 150ºC, pH 6.80-7.05, dissolved oxygen 6.5-7.2 mg/L, free carbon dioxide 4.5-6.5 mg/L, bicarbonate alkalinity 105-109 mg/L.

2.3. Haematological study

After acclimatization fishes were kept in two groups, 20 fish in each group, group 1 served as control. Fishes in group 2 were exposed to sublethal concentration of Mancozeb, 80% of LC_{50} of 24h at 24 h intervals for one week. Approximately 2mL of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA) an anticoagulant; it was properly mixed and used for haematological analysis. All haematological parameters such as red blood cell (RBC) count, the total white blood cell count, the hemoglobin (Hb) concentration and the packed cell volume (PCV), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were estimated by an auto analyzer SysmexR fully computerized automatic blood cell counter (KX-21).

2.4. Genotoxic study by means of micronucleus assay

After acclimatization fishes were kept in two groups 10 fish in each group, group 1 served as control. Fishes in group 2 were exposed to sublethal concentration of Mancozeb, 80% of LC_{50} of 24h for 24, 48, 72 and 96 h. After 24, 48, 72 and 96 h, peripheral blood of one fish from each group was collected by caudal vein and micronuclei assay were performed. Micronuclei are some extra nuclear bodies that are formed in mitosis from acentric chromosomal fragment or chromosomes that are not included in either daughter nucleus. Micronucleus assay is widely used in fishes since, it is simple economic and reliable technique to detect effect of chemical. After exposure the peripheral blood was collected from caudal vein or by heart puncture. A smear was prepared on clean glass

slide. The smear was fixed with methanol for 5 min, air dried and stained with 2% Giemsa. The slides were scanned and analysed for 1000 cells/individual with micronuclei.

2.5. Statistical analysis

Results were expressed as mean ± SE of three replicates and differences between means were considered to be significant when $p < 0.05$.

3. RESULTS

3.1. Hematological observation

The mean values for the haematological parameters of *Clarias batracus* were studied. The haematological parameters for the treated fish and those of the fish from the control tanks after one week were observed. The blood parameters of clarias batracus, namely the haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell (RBC) levels significantly decreased, whereas the white blood cell (WBC) level increased and no significant differences is observed in mean corpuscular volume (MCV) with exposure to the mancozeb (Table 1). No significant changes were observed in the measured variables of the control fish ($P > 0.05$).

3.2. Frequency of micronuclei

The frequencies of micronuclei induced in the peripheral blood erythrocytes, determined at concentration of 2.2 mg/l of mancozeb are shown in Table 2. The numbers of micronuclei at 48 h were maximum (0.5% 24h – 0.8% 48h) and thereafter decreased from 72h to 96h (0.5% 72h – 0.2% 96h) than the control fish.

Table 1. Changes in the hematological parameters of *Clarias batracus* exposed to mancozeb at a 24 h interval for 1 week.

Parameters	Control	Mancozeb
RBC (106 mm^3)	2.81 ± 0.23	2.11 ± 0.13
Haemoglobin (gdL^{-1})	9.06 ± 0.05	7.35 ± 0.01
MCV (µg)	91.23 ± 0.09	91.19 ± 0.05
MCH (Pg)	33.03 ± 0.13	28.31 ± 0.35
MCHC (g/dL)	38.16 ± 0.05	33.17 ± 0.19
WBC (103 mm^3)	1.43 ± 0.81	1.95 ± 0.17

Significance at 0.05 levels.

Table 2. Frequency of micronuclei in 1000 blood cells of *Clarias batracus* treated with mancozeb at different duration.

Duration of exposure	Slide No.	No. of blood cell examined	Total No. of cell with micronuclei		% of cell with micronuclei	
			Control	Mancozeb	Control	Mancozeb
24h	1	1000	0	5	0%	0.5%
	2	1000	1	7	0.1%	0.7%
48h	1	1000	0	9	0%	0.9%
	2	1000	1	8	0.1%	0.8%
72h	1	1000	1	5	0.1%	0.5%
	2	1000	0	4	0%	0.4%
96h	1	1000	0	3	0%	0.3%
	2	1000	0	2	0%	0.2%

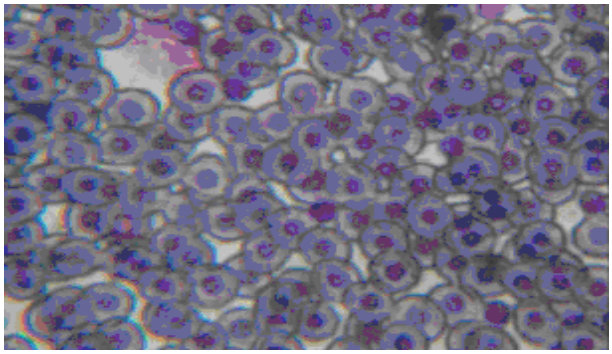

Figure 1. Blood cells of *Clarias batracus* without any treatment.

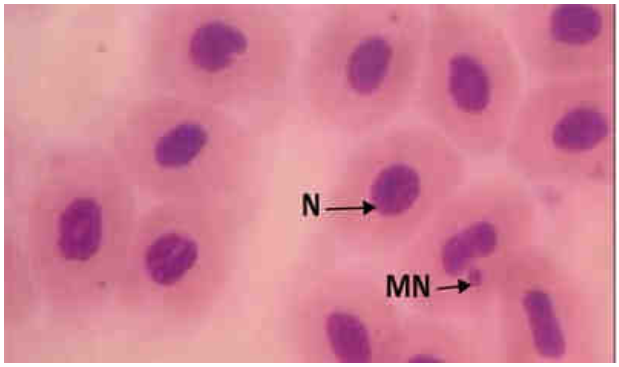

Figure 2. Peripheral blood smear showing normal nucleus (N) and micronucleus (MN) after exposure of mancozeb.

4. DISCUSSION

Significant decreases occurred in Hb levels after exposure to a sublethal dose, which may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production [23]. The significant decrease in the Hb concentration may also be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis [16]. Similarly, in freshwater catfish (*H. fossilis*), the Hb (%) decreased after 30 days exposure to deltamethrin [24]. The decreased RBC count may be due to inhibited RBC production and or due to hemoglobin synthesis, Ambient- toxicants might have caused disintegration of RBC cells, which in turn have caused reduction of hemoglobin and hematocrit count. The MCHC is a good indicator of red blood cell swelling [25, 26]. The significant decreased in the MCHC is probably an indication of red blood cell swelling and/ or to a decrease in hemoglobin in hemoglobin synthesis [27]. WBC count increase, reflecting the occurrence of leukocytosis (WBC increase). This was perhaps, a typical defensive response of the fish against a toxic invasion and second most common probability may be leukemia or blood cancer during which the number of WBC increase. In another study, Lohner et al. [28] reported leucopenia (reduced WBC counts) in sunfish inhabiting selenium laden coal ash effluents and associated it with increasing liver metal concentations. This is in agreement with the findings of Sampath et al. [29] when they exposed the Nile tilapia *O. niloticus* to a toxic environment. Finally it is concluded that the fish hematological parameters such as HB, RBC and MCHC are decreased showing anemia conditions, where as the WBC count is increased. Toxicants caused hyperproteinaemia, hyperlipaemia, hypoglycemia and hypochalesterolemia.

Apparently the action of any chemical genotoxic agent may give rise to an increase in micronucleus frequency. Consequently, based on the fact that spontaneous formation of micronuclei is normally low and nearly uniform among species [30], in environmental monitoring, micronucleus assaying has emerged as a simple, inexpensive and rapid method for detecting genotoxic effects. In our study, significant frequencies were observed in the treated fish than control fish. The present study signifies the effect of mancozeb on an economically important food fish *C. batracus* in terms of micronuclei test. The frequency of micronuclei in the present study significantly ($p<0.05$) increased from 0 to 48 h post treatment. Reports on the impact of mancozeb addressing micronuclei in *C. batracus* are not available. However, Rishi and Grewal [31] reported that micronucleus assay results show constancy of effect over various duration in dichlorvos on *Channa punctatus*. According to Kirsch-Volders et al. [32], MN assay is a multi endpoint test of genotoxic responses to clastogens. The frequency of micronucleus (MN) is extensively used as a biomarker of genomic stability [33]. Micronuclei result from chromosome breakage or interference with the mitotic apparatus and such events are thought to be related to carcinogenesis [34]. In the present study, total number of micro-

nuclei initially increased up to 48 h of exposure thereafter decreased at 72 or 96 h of exposure. Remirez and Saldanha [35] and Yadav et al. [21] demonstrate that this decrease is due to its effect in the form of broken egg and appearance of multiple micronuclei which are considered to be associated with severe chromosomal aberration. Since, fishes can respond to mutagens at low concentration of toxicant in a manner similar to higher vertebrates [36, 37]. Moreover, as compared to mammals, the DNA repair was reported to be slower in fishes [38-40].

5. CONCLUSION

These studies clearly reveal the genotoxic and haematological potential of mancozeb. Therefore, these investigations suggest a serious concern towards the potential danger of mancozeb to aquatic organisms and its use in agriculture practices.

AUTHORS' CONTRIBUTION

SC: conception and design; AS: development of methodology; SC: acquisition of data; SC and AS: analysis and interpretation of data; SC and AS: writing, review and revision of manuscript; administrative, technical, or material support; AS: study supervision. All authors are involved in drafting the manuscript, read and approved the final manuscript.

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The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Traditional knowledge on wild edible plants as livelihood food in Odisha, India

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ABSTRACT

An ethnobotanical investigation was carried out in the interior of Kendrapara district, Odisha, India to explore the potential use of edible plants by local inhabitants. Two hundred and twenty three informants of various ages in different villages of the site provided information on plant species used as food. Information on the use of edible plants was obtained through structured questionnaires, complemented by free interviews and informal conversations. The study documented 86 edible plant species belonging to 51 families. Amaranthaceae, Dioscoreaceae and Caesalpiniaceae were the most important botanical family**.** Of the reported species trees and herbs make up the highest proportion of the edible species comprising 38.4% and 20.0 % respectively. Within the edible plant parts, leaves and fruits contributed 35.4% and 33.3% and the remainders were edible tubers, flowers and seeds. Present study demonstrated that there is an urgent need for documentation of traditional knowledge related to the intangible cultural heritage concerning traditional plant uses. The utilization and cultivation of these vegetables should be promoted to maintain the dietary needs of the household in Odisha. The study can provide a baseline data that may be helpful for prioritization of conservation through sustainable use and management of the resources.

Key words: Edible plants; Ethnobotany; Kendrapara district; Odisha; Traditional knowledge.

INTRODUCTION

Human health depends on the quality of the environment in which people live. The interrelationships between society and nature, and the importance of environment health to human health depend on biodiversity which have direct impact on human-well being as well. Despite the primary reliance of agricultural societies on domesticated plants and animals for food, the tradition of consuming wild plants has not been completely erased. Millions of people, particularly tribal and rural communities in many developing countries still collect and consumed a wide variety of wild plant resources to meet their food requirements [1-3]. Wild sources of food, in general, remain particularly important for the poor and landless, and are especially important during times of famine or conflict when normal food supply mechanisms are disrupted and local or displaced populations have limited access to other kinds of food. The role of these edible plant species in maintaining human and environmental health has been reported [4, 5]. Intensive studies concerning its nutritional role have also been highlighted in many surveys around the world [6-13]. Moreover, these plants have played an important role in complementing staple foods to provide a balanced

diet by supplying of protein, fat, sugars, trace elements, vitamins, and minerals. This topic is relevant at the moment, as biodiversity conservation and its links with nutrition and human health is the subject of a recent cross-cutting initiative by the Convention on Biological Diversity [14]. Presently, a considerable proportion of rural population, particularly in remote areas of India do not produce enough food grains to meet yearly food requirement. Therefore, a large share of rural population is meeting their nutritional requirement through unconventional means, by consuming various wild plants [15]. Although only three crops -wheat, maize and rice provide around 50% of human energy intake [16]; some 7000 species are used, or have been used, for human food [17].

Traditional knowledge on the edible plants has been developed and used in virtually all cultures [13, 18-22] around the globe and India is no exception to it. The traditional knowledge and consumption of wild edible plants of Indian ethnic communities is rich and unique in the world [23-29]. Odisha, one of the eastern states of India has oldest and richest cultural traditions of using plants for various ethnobotanical purposes. Its diverse topography has permitted the survival of traditional knowledge related to plant resources used by locals as food. Even though, the consumption of plants gathered from the wild represented an important part of human nutrition in Odisha, however, a perusal of literature reveals that except some studies by Sinha and Larka [30] and Rout [31] on the wild edible plants the state remains largely unexplored. In this context, there have, however, been no reports in Kendrapara district of Odisha, India. The rapidity with which environmental damage, loss of floristic and cultural diversity occurs today, a necessicity is felt for the recording and documentation of traditional knowledge about the uses of edible plants - knowledge which is widely disappearing. Therefore, there is an urgent necessity to document traditional knowledge, focusing on the maintenance of this important cultural practice. It seems therefore imperative to investigate the current available knowledge on wild edible plants traditionally used in Kendrapara district of Odisha, India during 2010-2012. The study can provide a baseline data on the value of such locally produced food source particularly at times of food shortage which may be helpful for prioritization of conservation through sustainable use and management of the resources.

2. MATERIALS AND METHODS

2.1. Study Area:

Kendrapara district (20^o 21' - 20^o 47' N and 86^o 14'- 87^o 03'E) is situated in central coastal plain zone of Odisha state, India (Fig. 1C) and covers an area of 2644 km^2 with a population of 1.302 million (2001 Census). It is bordered by Bhadrak district in the north, Jagatsinghpur in the south, Bay of Bengal in the east and Cuttack and Jajpur district in the west. The district accounts for 1.61% of the state's territory and shares 3.62% of the state's population. Most of its people live in villages (94.2%) and agriculture is their main occupation. The district is also known for famous crocodile sanctuary at 'Bhitarkanika National Park' and Gahirmatha Sanctury for *Olive ridley*, besides for numerous ancient shrines like "Baladevjew (Lord Balabhadra) and "Pancha Barahi" (the five mothers).The climate of the study area is warm and humid. Three distinct seasons are felt during a year; rainy (mid June to October), winter (mid October to February) and summer (March to mid June). The air temperature ranges from 38°C in summer to 13°C in winter with an annual average 1500 mm rainfall. The district is located in the deltaic region with close proximity to the Bay of Bengal. Obviously, it has all the features of a costal climate i.e. saline weather, influence of coastal wind and cyclone proneness.

2.2. Data Collection:

The method employed in this study were designed with the purpose of providing base line information on the use of plant species in local system, through literature survey and field visits to various areas from November 2010 to December 2012 in the Kendrapara district, Odisha. Ethno-botanical information on the use of food plants was obtained through a combination of tools and techniques of structured questionnaires, complemented by free interviews and informal conversations [32]. During the field survey- aims, methods, anticipated benefits of the study were

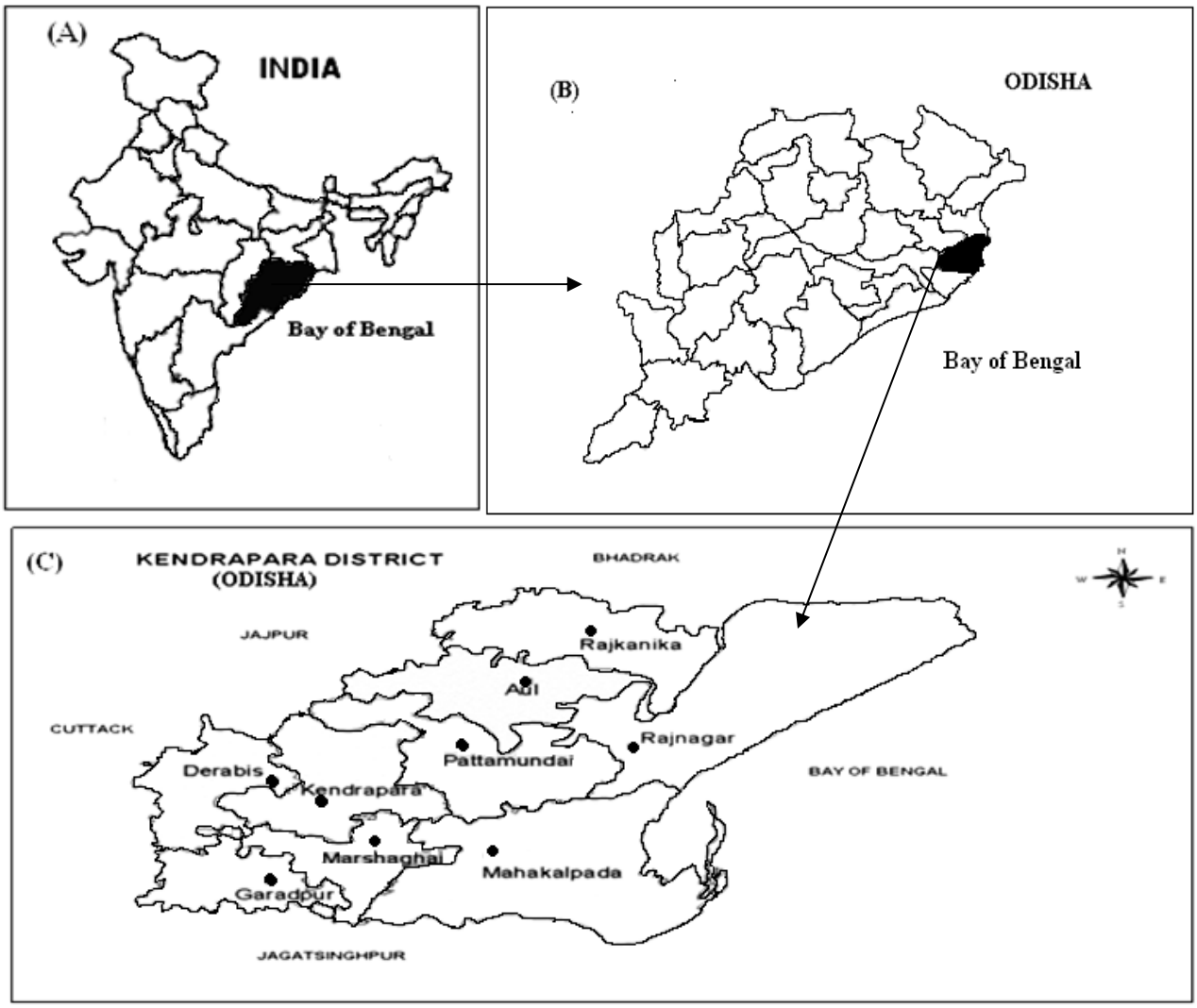

Figure 1. (A) location of the Odisha state in the eastern region of India, (B): map of Kendrapara district in central coastal plain zone of Odisha state, and (C) study area showing nine blocks of the Kendrapara district.

adequately informed to the informants and due consent has been taken from the local people in this regard to publish the information obtained from them. The interviews were individually carried out with members of the local population. Most of the selected informants belong to those families who have a strong connection with traditional agriculture for their day-to-day needs. In most cases selection of informants were based on recommendations made by local community members on those elders who were more knowledgeable about the use of local flora. Number of respondents, their age class, gender structure, localities visited and geographical features of each block of the Kendrapara district is depicted in Table1 and 2. Two hundred and twenty three (122 men and 101 women) persons were interviewed. Among the interviewees, 10% were of age 21–40 years, 40% were 61 years old or more and 50% were of age of 41–60 years.

Surveys were conducted in various villages of different blocks (*viz*., Aul, Derabis, Garadpur, Kendrapara, Mahakalpada, Marshaghai, Potta-mundai, Rajkanika and Rajnagar) of the district (Fig. 1C). Collections are valuable because they serve as voucher specimens, and records of the plants [33]. A voucher specimen facilitates the identification of the species encountered during the research and permits colleagues to review the results of the study [34, 35]. Informants with a sound traditional knowledge of useful wild plants, mostly elderly long-time residents, were consulted to record local plant names, edible parts, habit and habitat, fruiting period and mode of utilization.

Table 1. Number of informants, localities visited and geographical features of each area of the Kendrapara district.

Survey sites	Informants	Localities	Surface Km^2	Population (2001 census)
Aul	29	12	224.45	136297
Derabis	27	08	171.4	129532
Garadpur	18	08	141.54	98297
Kendrapara	22	11	260.63	178919
Mahakalpara	31	14	490.57	191745
Marsaghai	19	08	159.65	115103
Pattamundai	27	10	261.2	179924
Rajkanika	29	09	263.49	126887
Rajnagar	21	13	346.25	145301

Table 2. The age class, gender structure and number of respondents from nine blocks of the Kendrapara district.

Block	Sex	Age-class					
		21-40		41-60		61+	
Aul	M	2	3	9	14	5	12
	F	1		5		7	
Derabis	M	1	3	8	14	6	10
	F	2		6		4	
Garadpur	M	1	2	3	8	4	8
	F	1		5		4	
Kendrapara	M	0	1	5	12	4	9
	F	1		7		5	
Mahakalpada	M	2	4	8	14	9	13
	F	2		6		4	
Marshaghai	M	1	1	5	9	6	9
	F	0		4		3	
Pottamundai	M	1	3	9	13	7	11
	F	2		4		4	
Rajkanika	M	2	4	9	16	4	9
	F	2		7		5	
Rajnagar	M	1	2	6	12	4	7
	F	1		6		3	
Total (%)	M	4.92	10.3	27.8	50.2	22.0	39.5
	F	5.38		22.4		17.5	

Personal interviews and group discussions with local inhabitants revealed some valuable and specific information about the plants that were authenticated by crosschecking. In addition to crosschecking and recording folk names of plants through collecting voucher specimens, it is important to crosscheck information with different people and compare the results from different methods [36]. Interviews with people out of the village (in pastures or forests) were conducted on a systematic basis to know more details about species, their management and distribution. The voucher specimens of plants were identified by using standard floras and available literature [37, 38]. The identified voucher specimens were deposited at the Chandbali College herbarium. The list of edible plants was depicted along with their botanical names followed by family name, their local names in Oriya, if any, and the parts used for food purpose.

3. RESULTS AND DISCUSSION

Investigation on the natural and traditional relationship between plants and human societies has brought to light several little or unknown uses of plants developed through trial and error method as well as by the creative mind of the indigenous people all over the world. Similarly various edible plants used by the local people of Kendrapara are one such typical use of the plant. Although the alluvial soil of the Kendrapara district is highly productive, it is prone to sea borne cyclonic sea storms and resultant flooding of saline as well as heavy rain water leading to failure of crops in alternate year. To combat such adverse climatic conditions and consequent shortage of food grain people use edible plant resources at least to sustain 2-3 months in a year. A total of 86 plants belonging to 51 families are recorded (Table 3; Fig. 2-5).

Dioscoreaceae and Amaranthaceae were the most frequently encountered botanical family with 6 species, whilst Caesalpiniaceae follow with five species. The reported plant species are gathered from natural environments, particularly in the areas surrounding villages, field crops, gardens, forest, road side and fallow land. Majority (87.2%) of the edible plants mentioned are the wild (Fig. 6). Some of the edibles such as *Artocarpus heterophyllus* Lamk., *Amorphophallus paeonifalius*, Dennst., *Bauhinia purpurea* L., *Bauhinia retusa* Roxb., *Colocasia esculenta* L., *Emblica officinalis* Gaertn., *Mangifera indica* L. and *Moringa oleifera* Lam. were domesticated in home gardens by local people as well as available in the wild. The data of this study shows that people tend to use preferably the plants that are easily available to them excluding of course, those that are toxic.

Figure 2. *Asparagus racemosus* Wild.

Figure 3. *Lantana camara* Linn.

Figure 4. *Mullugo pentaphylla* Linn.

Figure 5. *Oxalis corniculata* Linn.

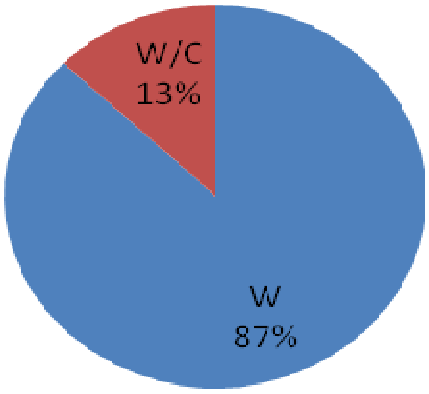

Figure 6. Distribution of wild and cultivated wild edible plants in Kendrapara district, Odisha.
W = Wild, W/C = Wild cum cultivated.

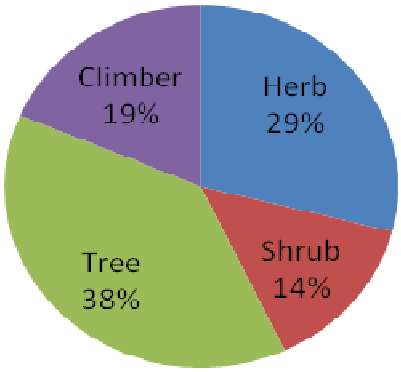

Figure 7. Growth forms of wild edible plants reported in Kendrapara district, Odisha.

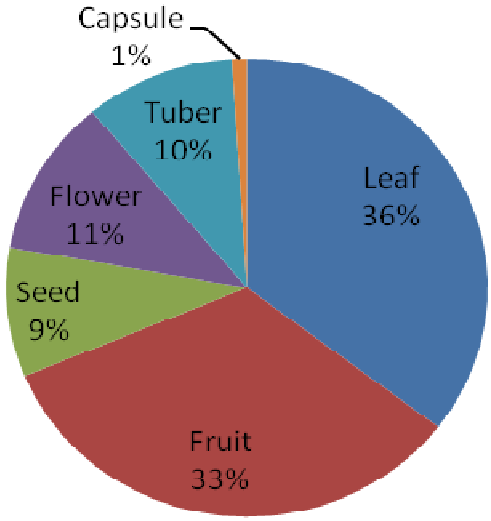

Figure 8. Classification of wild edible plant parts reported in Kendrapara district, Odisha.

Table 3. Traditionally consumed edible plants of Kendrapara district, Odisha.

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Asteracantha longifolia L. Koelekha	Acanthaceae	Wild, a wasteland shrub.	Leaves are cooked as vegetable.	08	+	Common
Trianthema portulacastrum L. Kachoa	Aizoaceae	Wild, a succulent herb and a weed.	Leaves are used as vegetable.	17	++	Occasional
Alangium salvifolium L. Ankula	Alangiaceae	Wild, small bushy tree in wasteland. Fl.Mar-Apr. Fr. June-July.	Ripen fruit pulp is eaten.	23	++	Occasional
Colocasia esculenta L. Saru	Araceae	Wild/cultivated, herb	Leaves and tubers are cooked as vegetable.	139	++++	Common
Amorphophallus paeonifalius, Dennst.Olua	Araceae	Wild/cultivated, herb	Tubers are cooked as vegetable.	137	++++	Common
Calamus rotang L. Beta	Arecaceae	Wild, tall climber	Ripen fruits are edible.	05	+	Rare
Phoenix sylvestris Roxb. Khajuri	Arecaceae	Wild, an unbranched wasteland tree. Fl. and Fr. Oct-May.	Ripen fruits are edible.	156	++++	Common
Buchanania lanzan Spreng. Charkoli	Anacardiaceae	Wild, a small tree in forests. Fl. Jan.-Mar. Fr. Apr.-May	Ripen fruits are edible.	79	++	Occasional
Mangifera indica L. Amba	Anacardiaceae	Wild/cultivated, tree. Fl. Jan-Mar., Fr.Apr.-May.	Unripe fruit is eaten as chutney and pickles; also eaten after ripening.	203	++++	Common
Aerva lanata (L.) Juss. Paunsia	Amaranthaceae	Wild, perennial herb and a weed.	Leaf and leafy shoots are cooked with mustard oil.	65	++	Occasional
Celosia argentea L. Lahenga	Amaranthaceae	Wild, a roadside weed.	Leaf and leafy shoots are cooked as vegetable.	54	+	Common
Alternanthera philoxeroides Mart.Grises Ghoda madaranga	Amaranthaceae	Wild, prostrate herb and a weed.	Leaf and leafy shoots are cooked as vegetable.	28	++	Common
Alternanthera sessilis (L.)R.Br. Madaranga	Amaranthaceae	Wild, prostrate herb and a weed.	Leaf and leafy shoots seasoned with mustard oil.	165	++++	Common
Amaranthus spinosus L. Kanta leutia,	Amaranthaceae	Wild, erect glabrous branched herb in cultivated and waste ground.	Leaf and leafy shoots are cooked as vegetable.	48	++	Occasional
Amaranthus viridis L.Marsi	Amaranthaceae	Wild, herb and a weed.	Leaf and leafy shoots are cooked with mustard oil/ mustard seed paste.	129	++++	Common

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Centella asiatica L. Thalkudi	Apiaceae	Wild, a herb in wet places.	Leaves and leafy shoots are eaten as cooked vegetable or chopped into pieces for making chutney.	91	+++	Common
Holarrhena antidysentirica Wall.exA.DC. Kurchi	Apocynaceae	Wild, a moderate sized tree species in wasteland.	Flowers are used as vegetable.	34	+	Rare
Garuga pinnata Roxb. Kathakusum,	Burseraceae	Wild, tree, Fl. Feb. - Apr, Fr. Jun.-Aug.	Ripen fruits are edible.	25	+	Rare
Bauhinia purpurea L.Debakanchan	Caesalpiniaceae	Wild/cultivated, a moderate sized tree species.	Flowers are eaten as vegetables.	69	++	Occasional
Bauhinia retusa Roxb. Choari	Caesalpiniaceae	Wild/cultivated, a moderate sized tree species.	Flowers are used as vegetable.	54	++	Occasional
Bauhinia variegata L. Kanchana	Caesalpiniaceae	Wild, a medium sized tree species.	Flowers are used as vegetable.	102	+++	Common
Cassia tora L. Chakor	Caesalpiniaceae	Wild, under shrub and a weed in wasteland.	Leaves are used as vegetables.	31	+	Rare
Tamarindus indica L. Tentuli	Caesalpiniaceae	Wild, tree, Fl. Apr.-Jun.,Fr. Dec.-Mar.	Ripen and raw fruits are eaten. Leaves and flowers are cooked as vegetables; Seeds are eaten in raw and roasted.	131	++++	Common
Capparis zeylanica L.Asadhua	Capparaceae	Wild, a Climbing shrub. Fl.Feb.-Apr. Fr. Sept.-Oct.	Ripen fruits are edible.	17	+	Rare
Cleome monophylla L. Rangasorisa	Capparaceae	Wild, under shrub in fallow fields.	Leaf and leafy shoots are cooked with mustard oil.	21	+	Rare
Cleome viscosa L. Anasorisia	Capparaceae	Wild, an erect herb and a weed.	Leaves are eaten as vegetables.	54	++	Occasional
Chenopodium album L. Bathua	Chenopodiaceae	Wild, herb.	Leaf and leafy shoots are cooked as vegetable.	117	++++	Common
Celastrus paniculata wild. Kujri	Celastraceae	Wild, a climbing shrub.	Flowers are eaten as vegetable.	16	+	Rare
Terminalia bellerica Gaertn. Bahada	Combretaceae	Wild, tree, Fl. March-May, Fr. Oct-Dec.	Seeds are sundried and made into flour for use.	37	+	Rare
Commelina benghalensis L. Kansiri	Commelinaceae	Wild, a weed in wet places.	Leaf and leafy shoots are cooked as vegetable.	29	++	Common

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Erycibe paniculata Roxb. Joraikoli	Convolvulaceae	Wild, a climbing shrub.Fl. May-June, Fr. Following Mar.-May.	Ripen fruit is sweet in taste.	141	++++	Common
Garcinia cowa Roxb.Rajkusuma	Clusiaceae	Wild, a tree species. Fl. Mar. - Apr, Fr. May-June.	Ripen fruits are edible.	62	++	Occasional
Mesua ferrea L. Nageswar	Cluslaceae	Wild, a forest tree. Fl. Mar.-July,Fr.Oct.-Nov.	Ripen fruits are eaten.	15	+	Rare
Dioscorea glabra Roxb. Kanta alu	Dioscoreaceae	Wild, climber	Tubers are eaten as vegetable.	76	++	Occasional
Dioscorea sativa Thunb. Pita alu	Dioscoreaceae	Wild, climber	The tubers are bitter; they lose their bitterness on roasting and are then eaten.	23	+	Rare
Dioscorea daemona Roxb. Bainya alu	Dioscoreaceae	Wild, climber	Tubers are eaten as vegetable.	13	+	Rare
Dioscorea oppositifolia L. Pitil kanda	Dioscoreaceae	Wild, climber	Tubers are edible.	47	+	Rare
Dioscorea pentaphylla L. Karba,	Dioscoreaceae	Wild, climber	Tubers are edible.	10	+	Rare
Dioscorea wallichi Hook.f.Tunga alu	Dioscoreaceae	Wild, climber	Tubers are edible.	49	++	Common
Shorea robusta Gaertn. Sal	Dipterocarpaceae	Wild, a timber yielding species, Fl. Mar.-Apr, Fr. May-June.	Raw fruit and seed are used as vegetable.	61	++	Occasional
Diospyros malabarica Desr. Dhusara kendu	Ebenaceae	Wild, a handsome tree, Fl and Fr. Mar.-Apr.	Ripen fruit is edible.	43	+	Rare
Diospyros melanoxylon Roxb. Kendu	Ebenaceae	Wild, a small tree found in wasteland. Fl Apr.-May ripens following May.	Ripen fruit is edible. Seed is eaten as roasted.	79	++++	Common
Emblica officinalis Gaertn. Aonla	Euphorbiaceae	Wild/cultivated, moderate sized tree in plains and hills. Fl. Feb.-May, Fr. Oct.-Apr.	Raw and ripen fruits are eaten. Jams and pickles are prepared.	162	++++	Common
Euphorbia hirta L. Chitakuti	Euphorbiaceae	Wild, prostrate hairy herb and a weed.	Leaves are eaten as vegetable.	25	++	Common
Abrus precatorius L.(V.Gunj) Kaincha	Fabaceae	Wild, shrub	Leaves are sweet testing and chewed as mouth freshener.	34	++	Common

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Casearia graveolens Dalz. Benchi	Flacourtiaceae	Wild, a small tree. Fl. Feb.-March Fr.Apr.-Jul.	Ripen fruits are edible.	07	+	Occasional
Asparagus racemosus .Wild. Satabari	Liliaceae	Wild, shrub	Tuberous root are edible.	54	+++	Common
Leucas aspera Wild. Gayas	Lamiaceae	Wild, a weed in roadsides.	Leaves are eaten as vegetable.	09	+	Common
Curculigo orchioides Gaertn. Talamuli	Hypoxidaceae	Wild, herb	Tuberous roots are edible.	76	++	Common
Abutilon indicum L.Sweet Pedipedica	Malvaceae	Wild,undershrub in waste places, as weed in gardens	Leaves are cooked as vegetable.	45	+	Common
Sida cordata Burm.f Bisiripi	Malvaceae	Wild, a weed herb.	Leaves are eaten as vegetable.	11	+	Common
Melia azadirachta L. Limba	Meliaceae	Wild, an aromatic tree of great folklore, considered sacred and a temple yard plant.	Flowers are cooked as vegetable.	76	++++	Common
Adenanthera pavonina L. Manda Kaincha	Mimosaceae	Wild, a tree found in gardens and village sides	Seed is eaten raw.	32	++	Common
Entada rheedii Spreng. Gila	Mimosaceae	Wild, A climber in wasteland.	Endosperm and seed is cooked with rice.	04	+	Common
Mullugo pentaphylla L. Pita gahama	Molluginaceae	Wild, a weed in cultivated land, road sides and wasteland	Leaves are cooked as vegetable.	143	++++	Common
Artocarpus heterophyllus Lamk. Panas	Moraceae	Wild/cultivated, Tree	Seed roasted and used as vegetable. Raw fruits are eaten after cooking. Ripen fruits are eaten before they are quite ripe.	193	++++	Common
Ficus hispida L.f. Dimiri	Moraceae	Wild, a wasteland tree species. Figs Nov.-July.	Ripen and raw fruits are eaten after cooking.	7	+	Common
Streblus asper Lour. Sahada	Moraceae	Wild, in village periphery and foot hill forests.	Flowers are used as vegetable. Ripen fruit is edible.	11	+	Common
Moringa oleifera Lam. Sajana	Moringaceae	Wild/cultivated, a medium sized tree.	Leaf, flower, capsule is used as vegetable.	186	++++	Common
Syzygium cuminii L. Jamu	Myrtaceae	Wild, A tree species. Fl.Apr.-May,Fr. Jul.-Aug.	Ripen fruits are largely eaten.	139	++++	Common
Boerhavia chinensis L. Puruni	Nyctaginaceae	Wild, a herb in village hedges.	Leaves are cooked as vegetable.	17	+	Occasional

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Boerhavia diffusa L.Ghodapuruni	Nyctaginaceae	Wild, a weed herb.	Leaves are used as vegetable.	27	++	Common
Oxalis corniculata L. Ambiliti	Oxalidaceae	Wild, prostrate herb, a weed in gardens and wasteland.	Leaves are used as vegetable.	79	+++	Common
Passiflora foetida L.Bisripi	Passifloraceae	Wild, a climber in wasteland and also planted in hedges. Fl. and Fr. Nov.-June.	Ripen fruit is eaten.	23	+	Common
*Bambusa ba*mboo L. Baunsa	Poaceae	Wild, Shrub	Seeds made into flour and are used in cakes.	14	+	Common
Polygonum plebeium R.Br. Muthisaga,	Polygonaceae	Wild, prostrate herb and a weed in moist places.	Leaves are cooked as vegetable.	167	++++	Common
Portulaca oleracea L. Bada balbalua	Portulacaceae	Wild, prostrate herb. A weed of cultivated land, wasteland and road sides.	Leaves are used as vegetable.	23	++	Rare
Portulaca quadrifolia L Balbalua	Portulacaceae	Wild, a creeping herb in cultivated land.	Leaves are used as vegetable.	37	++	Occasional
Ziziphus rotundifolia Lamk. Tinkoli,	Rhamnaceae	Wild, a small branched shrub.Fl. Oct.-Dec.,Fr.Nov.-Feb.	Ripen fruit is edible.	76	++	Common
Ziziphus oenoplia L. Mill.Kanteikoli	Rhamnaceae	Wild, a thorny climbing shrub in wasteland. Fl. Jun.-Sept.,Fr.Oct.-Jan.	Ripen fruits are eaten.	142	++++	Common
Ziziphus jujuba L. Barokoli, Rhamnaceae	Rhamnaceae	Wild/ cultivated branched thorny species.Fl. Mar.-Oct., Fr.Jan.-Mar.	Unripe fruit is eaten as pickles and also eaten after ripening.	207	++++	Common
Ixora undulata DC. Karuna	Rubiaceae	Wild, a large shrub in coastal plains and hills. Fl. Apr.-May, Fr.Aug.-Sept.	Ripen fruits are edible.	18	+	Common
Aegle marmelos Corr. Bel	Rutaceae	Wild/cultivated tree species. A plant of great folklore, considered sacred and a common temple yard plant. Fl.March-Apr. Fr. after one year.	Fruits become yellow when ripe. Sweaty pulp is eaten raw or prepares soft drink.	199	++++	Common

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Murraya koenigii L. Spreng. Bhursunga	Rutaceae	Wild, tree	Leaves are eaten as ingredient of curry.	176	++++	Common
Swietienia chloroxylon Roxb. Bheru	Rutaceae	Wild, small tree planted in the gardens and roadsides.	Leaves are used as vegetables.	47	++	Occasional
Madhuca indica J.F. Gmel. Mahua	Sapotaceae	Wild, a tree in forest and village sides.	Flowers and seeds are cooked as vegetable.	27	++	Occasional
Mimusops elegani L. Baula	Sapotaceae	Wild/cultivated, tree. Fl. Apr.-May, Fr.Aug.-Sept.	Ripen fruit is eaten.	133	++++	Common
Schleichera oleosa Lour. Kusum	Sapindaceae	Wild, a deciduous tree in coastal plains and hills. Fl. Feb.-Mar., Fr. Jun.-Aug.	Yellow pulp is eaten when ripe. Pleasant and acidic in taste. Seeds are also used as vegetable.	74	+++	Common
Smilax zeylanica L. Rajdantari	Smilacaceae	Wild, a medium sized climber. Fl. Apr.-Jul., Fr. Oct.- Jan.	Ripen fruit is edible.	16	+	Occasional
Solanum nigrum L. Lunikoli	Solanaceae	Wild, an erect branched herb common in waste ground.Fl. and Fr. Most of the year.	Ripen and raw fruits are edible.	31	++	Common
Solanum torvum Sw. Kathakoli	Solanaceae	Wild, shrub in wasteland.	Leaves are eaten as vegetable.	46	+++	Common
Solanum viarum Dunal in DC. Bhejibaigana	Solanaceae	Wild, an erect branched shrub common in waste land. Fl. and Fr. Most of the year.	Fruit is eaten as vegetable.	89	+++	Common
Sterculia urens Roxb. Genduli	Sterculiaceae	Wild, a moderate sized tree.	Seed is eaten as roasted.	19	+	Occasional
Dregea volubilis L.f. Dugdhica	Strychnaceae	Wild, common tree in coastal plains and forests. Fl. Apr.-June, Fr. Dec.-Feb.	Ripen fruits are edible. Flower is used as vegetable.	22	++	Common
Pouzolzia zeylanica L. Kupachera	Urticaceae	Wild, a herb in village hedges	Leaves are used as vegetables.	09	+	Common
Gmelina arborea Roxb. Gambari	Verbenaceae	Wild, moderate sized forest species. Fl. Mar.-Apr. - Fr. May-June.	Ripen fruits are edible.	13	+	Occasional

Botanical name, authors, local name	Family	Habit/habitat/ domestication	Parts used and mode of consumption	NP	Rank of use	Abundance
Lantana camara Linn. Naga airi	Verbenaceae	Wild, a wasteland weed.Fr and Fl. all the year around.	Ripen fruits are edible.	24	+	Common

Legends: NP: Number of persons who cited the species, + Rarely used; ++ Fairly used; +++ Moderately used; ++++ Extensively used.

Of the reported growth forms, trees and herbs make up the highest proportion of the edible species comprising 38.4% and 29.0% respectively (Fig. 7). Within the edible parts of the wild food plant, leaves (37.2%) and fruits (33.0%) were most widely used and the remainders were flower, tuber and seed (Fig. 8). The present report on the use of plants for food purposes draws support from earlier studies in different parts of India [23-26, 28, 29, 39-41]. Some of the studied plants are also frequently used in Dhenkanal, Keonjhar and Mayurbhanj districts of Odisha [30, 31]. When I compare the results with different countries of the globe I see that many of the Odishan edibles are frequently used although some of the recipes differ [6-13]. It was observed that the traditional knowledge about the use of wild edibles exist only in the collective memory of the elderly and face a slow and natural death; on the other hand the young generation relies on the vegetables and fruits that dominate the market and have poor knowledge about the wild edible plant. Many of these wild edible plant species are found to be sold in the local markets particularly by poor and economically marginalised families, thereby generating a supplementary income to their household economy.

Most wild fruits or flowers mentioned were consumed by children on the way to school, or when tending livestock. Some people still pick them on walks to relive the flavours of their childhood. Such types of observation have also been reported by several workers [2, 42-45]. A number of wild edible fruits were gathered, preserved, stored, and consumed some weeks or months after gathering. Most of the respondents employ sun drying as a means of food preservation. *Artocarpus heterophyllus* fruits for example, were sun dried and stored in plastic bottles or glass vessels and used several months after harvest. Some plants produce more than one edible product, such as *Tamarindus indica* which produces edible fruits, leaf, seed and flower; *Colocasia esculenta* produces edible leaf and tuber. Moreover, a fairly good number of these edible plants such as *Abrus precatorius, Aegle marmelos, Alternanthera sessilis, Asparagus racemosus, Centella asiatica, Moringa oleifera, Murraya koenigii, Syzygium cuminii* are also reported to have both therapeutic and dietary functions and hence are used as medicinal food remedy. This overlap indicates the close relationship between health and food. Overlapping between foods and medicines is quite well known in traditional societies [46, 47].

4. CONCLUSION

The results of this study have revealed that traditional knowledge on the use of edible plants is still practiced by the rural people in Kendrapara district, Odisha, India. The preservation of this knowledge is due to continued reliance on wild edible plants for consumption at times of food shortage and that these species have the potential to become valuable staple foods and important alternatives to the usual food crops by many households. Moreover, many of these edible plants of this district are rapidly shrinking due to population explosion and consequent human activities like construction of roads, housing, agricultural land expansion etc. and also lack of sustainable harvesting practices (destructive harvesting practice by locals as branches are lopped to collect fruit in a short time).It has thus been instrumental for documentation of traditional knowledge related to the intangible cultural heritage concerning traditional plant uses. Efforts to conserve biodiversity and preserve traditional food systems need to be combined and enhanced for the

benefit of the posterity. Further studies providing this data would greatly assist in promoting the involvement of local people in managing their resources.

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

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ORIGINAL ARTICLE

Toxicity of azadirachtin on some biomarkers of oxidative stress in zebrafish, *Danio rerio*

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ABSTRACT

Use of synthetic insecticides to control a wide range of pests has adverse environmental effects in addition to high operational cost. Insecticides of botanical origin have been reported to be useful for control of insect pests. Unfortunately, the indiscriminate use of these insecticides to improve agricultural production and yield may have impacts on non-target organisms, especially aquatic life forms. The present study was designed to investigate the impacts of Achook® (Azadirachtin 0.15%) on the stress biomarkers catalase (CAT) activity, malondialdehyde (MDA) and reduced glutathione (GSH) level in the brain and muscle of zebrafish, *Danio rerio*. After Achook® exposure fish showed a reduction in GSH level and CAT activity. The reduction in the CAT activity is probably related to the occurrence of increased lipid peroxidation (LPO) in fish exposed to the insecticide. The changes in the biochemical parameters were time as well as concentration dependent. It is concluded that a sub-lethal concentration of even natural pesticide may not be safe, it cannot be used indiscriminately. Therefore, pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic life as well as human beings.

Key words: Azadirachtin; Zebrafish; Antioxidants; Brain; Muscle.

INTRODUCTION

Extensive use of synthetic chemicals for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects and high operational cost. Large percentage of agricultural pesticide application never reaches its target organisms but is instead dispersed through air, soil and water [1]. Therefore, in considering the use of these chemicals, the benefits must be weighed against the risk to human health and environment. A major risk is environmental contamination, especially translocation within the environment where pesticides might enter the natural water systems and causes various deleterious effect on fish and ultimately on human.

Fish can serve as bio-indicators of environmental pollution and can play significant role in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem [2]. Teleost fish have proved to be good models to evaluate the toxicity and effects of contaminants on animals, since their biochemical responses are similar to those of mammals and of other verte-

brates [3]. The synthetic pesticides may reduce reproductive ability [4] and causes various biochemical alterations [5-9] in fishes. Natural pesticides based on plant extracts like azadirachtin have been commonly employed for pest control [10]. However, with the introduction of the synthetic organic chemicals, they lost their importance. Nowadays, azadirachtin, a biologically active compound of neem (*Azadirachta indica* A Juss) has been promoted as a new insecticide that is considered more eco-friendly than synthetic pesticides [11].

Fish are endowed with antioxidant defense system like catalase (CAT) and reduced glutathione (GSH) to counteract the impact of reactive oxygen species (ROS) resulting from metabolism of various synthetic chemicals. CAT is a common enzyme found in nearly all the organisms where it functions to catalyze the decomposition of H_2O_2 to O_2 and H_2O. GSH is an antioxidant which helps to protect the cells from the ROS. Oxidative stress develops when there is an imbalance between pro-oxidants and antioxidants ratio, leading to the production of ROS [12, 13]. ROS, such as hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\cdot-}$), and hydroxyl radical ($OH\cdot$) can react with biological macromolecules potentially leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and even cell death [14, 15]. Pesticide induced alteration in oxidative stress parameters due to free radicals has already been described for various fish species [16, 17] and considered as the main mechanism of cellular destruction. Recently, Modesto and Martinez [18] found that exposures to sub-lethal concentrations of a herbicide Roundup® altered antioxidant defenses in brain and muscle of *Prochilodus lineatus*.

In view of the above, and considering the lack of knowledge about the toxic potential of the neem pesticides to aquatic animals and the growing use of these pesticides, the objective of this work was to evaluate the effects of neem-based formulation Achook® on some biochemical parameters (CAT, GSH and LPO) of zebrafish (*Danio rerio*). Zebrafish was selected for the present study because they are model organisms for toxicological research and also recommended by International Organization for Standardization and the Organization for Economic Co-operation and Development [19].

2. MATERIALS AND METHODS

2.1. Test organism and treatments

An emulsified concentrate of neem kernel based formulation, Achook® (Azadirachtin 0.15%) manufactured by Bahar Agrochem & Feeds Pvt. Ltd., Maharashtra and marketed by Godrej Agrovet Ltd., Gujarat, India, was purchased from local market. For the present study, zebrafish, *Danio rerio* were procured from our stock aquarium and exposed to different concentrations of Achook® viz., 0.025, 0.17 and 0.35 µg/L i.e., 96-h LC_5, LC_{10} and LC_{20} respectively as calculated earlier using StatPlus® version 2009 computer software [7] for 16 days continuously. Low concentrations are selected keeping in view that the fishes may survive but under stress of toxicant. Six aquaria, with two replicates for each concentration of pesticide were used accompanied by their respective controls having equal quantity of acetone. Fifty fishes for each concentration of the pesticide were used. The water with toxicant was changed after every 24-h by adding the fresh pesticide solution in order to counterbalance decreasing pesticide concentrations due to its degradation in water. The experiment was conducted in glass aquaria containing 25-L of dechlorinated tap water. Temperature of water was maintained at 25 ± 2 ºC and aerated by mechanical compressor.

2.2. Tissue sample preparation

After the expiry of the exposure periods (4, 8, 12 and 16 days), required number of exposed fishes were taken out from experimental and control groups and their tissues (brain and muscle) were processed. Homogenates (prepared as 1 g tissue per 10 volumes of buffer) of the tissues were prepared in ice-cold buffer (0.1M Tris-EDTA buffer, pH 7.4) using a homogenizer. The resulting homogenate was centrifuged at 8,000g for 30 minutes in a refrigerated ultra centrifuge at 4 ºC. The clear supernatants collected were used for protein estimation and analysis of the biochemical parameters.

2.3. Determination of oxidative stress biomarkers

The levels of LPO were measured spectrophotometrically at 560 nm, via the thiobarbituric acid reacting substances (TBARS) colour reaction for malondialdehyde (MDA) according to the method of Placer et al. [20] and results were expressed as µM of MDA formed/mg protein. CAT (EC 1.11.1.6) activity was determined by monitoring the disappearance of hydrogen peroxide (H_2O_2) at 570 nm, according to the method of Sinha [21] and values were expressed as µM/minute/mg protein. GSH level was estimated according to the method of Paglia et al. [22] determined by its reaction with 5, 5'-dithio-bis (2-nitro-benzoic acid) (DTNB) to yield a yellow chromophore which was measured spectrophotometrically at 412 nm and results were expressed as GSH mg/mg protein.

2.4. Determination of protein contents

In tissues the total protein contents were assayed using the method of Lowry et al. [23], with bovine serum albumin as standard.

2.5. Statistical analysis

The data obtained was analyzed statistically by two-way analysis of variance (ANOVA). The differences between the control and the exposed groups were well checked. All data are expressed as means (n = 6) ± standard deviation (SD) and the criterion for significance was set at $P < 0.05$. Statistical analysis was performed by using StatPlus® version 2009 computer software purchased from Analystsoft Vancouver, Canada.

3. RESULTS

During the present investigation a significant ($P < 0.05$) alterations were observed in the CAT activity, GSH level and LPO in brain and muscle of zebrafish exposed to Achook® at different concentrations and exposure periods. The MDA contents significantly ($P < 0.05$) increased in the brain and muscle of zebrafish exposed to three different concentrations of pesticide for all exposure periods. The MDA concentration was increased to 161% in brain and 134% in muscle where controls were 100% after exposure to 96-h LC_{20} of the pesticide after 16 days (Fig. 1 and 2).

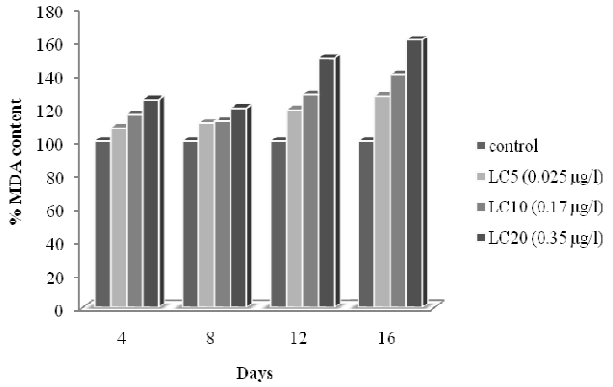

Figure 1. Effect of Achook on brain LPO in zebrafish.

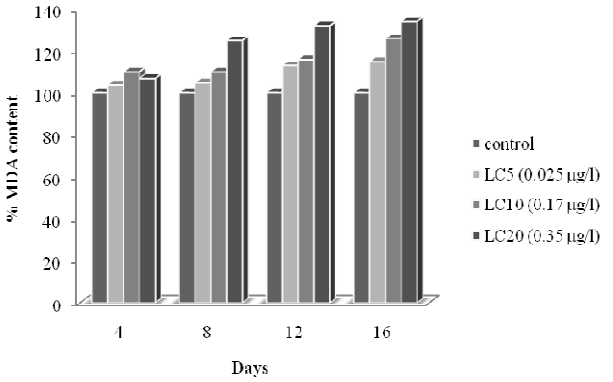

Figure 2. Effect of Achook on muscle LPO in zebrafish.

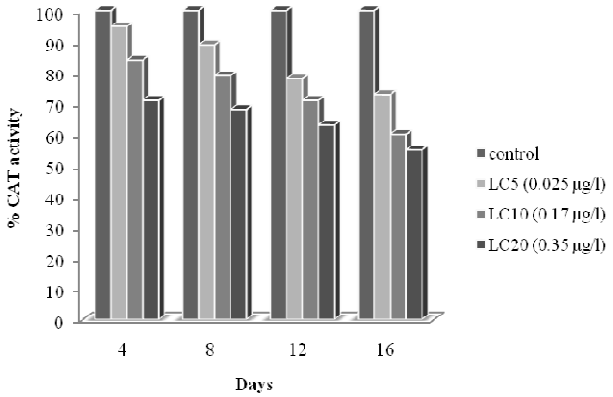

Figure 3. Effect of Achook on brain CAT activity in zebrafish.

Figure 3 shows the effects of Achook® exposure on the activities of CAT in zebrafish brain. The CAT activities decreased gradually with increase in treatment and exposure period. The significant ($P < 0.05$) change was recorded on the 16-day, when CAT activity was decreased by 55%

to 96-h LC_{20} of pesticide. Similarly, in the muscle, CAT activity was decreased, with the maximum on the 16-day (by 68%, compared with controls) at LC_{20} treatment of pesticide (Fig. 4).

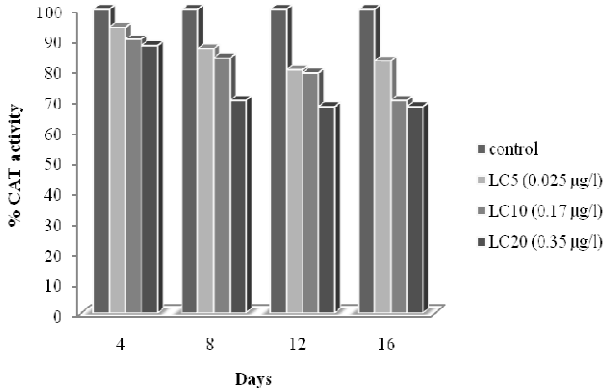

Figure 4. Effect of Achook on muscle CAT activity in zebrafish.

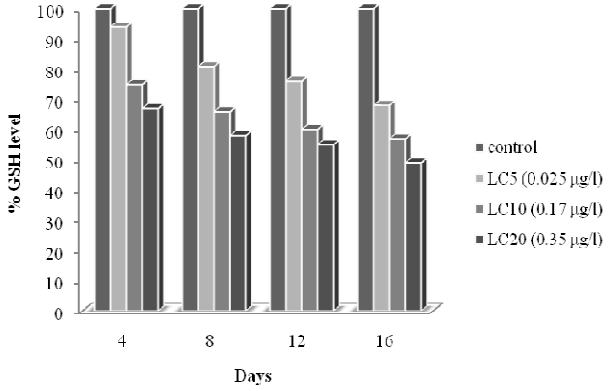

Figure 5. Effect of Achook on brain GSH level in zebrafish.

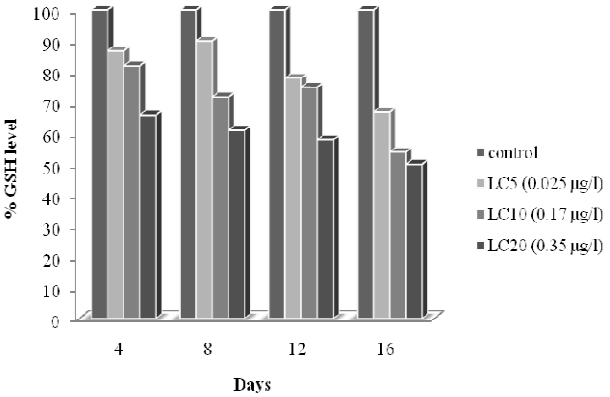

Figure 6. Effect of Achook on muscle GSH level in zebrafish.

Also, in the brain of zebrafish a significant ($P < 0.05$) decrease of GSH levels up to the 16 days pesticide exposure was observed (Fig. 5). In the muscle, a sudden decrease by approximately 61% of GSH was recorded on the 8-day post-exposure while by the end of the experimental tenure it decreased to approximately 50% of control at 96-h LC_{20} of the pesticide (Fig. 6).

4. DISCUSSION

Neem (*A. indica*) trees are found throughout India with a myriad of uses in medicine, as well as pest control [24, 25]. Neem pesticides are now extensively used in agriculture practices all over the world. These formulated pesticides and natural extracts have a wide range of effects against insect pests and are relatively safe towards non-target biota with only minimal risk of direct adverse effects on aquatic biota [26]. An emulsion of neem oil in water was found to be effective in controlling breeding of different species of mosquitoes in pools, tanks and coolers [27, 28]. A neem-based formulation containing 32% neem seed oil (0.03% azadirachtin) was investigated for its larvicidal activities against *Anopheles gambiae* [29].

Recently, toxic effects of a neem-based formulation in zebrafish were determined using behavioral models and concluded that it reduced the general activity and increased the anxiety-like behavior [30]. Also, the disturbance in the total protein level due to neem extracts have been reported [31].

During the present investigation it was observed that the neem-based pesticide Achook® promotes changes in the antioxidant defenses of brain and muscle of zebrafish, *D. rerio*. As for the antioxidant enzyme, the reduction in CAT activity after 16 days of exposure to all concentrations of the pesticide may be related to the increased production of ROS. According to Mekail and Sharafaddin [32] the activity of CAT was decreased in brain, liver and kidney of weanling rat intoxicated with Diazinon, Carbaryl and λ-Cyhalothrin. The superoxide dismutase (SOD) and CAT system is the first line of defense against oxygen toxicity, and these enzymes are used as biomarkers [33]. The concentration-dependent decrease in the activity of CAT observed in this investigation is in the

agreement with the report of earlier studies [34, 35, 36]. Ahmad et al. [12] explained the decreased CAT activity could be provoked by the flux of superoxide radicals ($O_2^{\cdot-}$) induced by the pollutants. Recently, Naveed and Janaiah [37] reported the decrease in CAT activity of brain, liver and kidney of *Channa punctatus* exposed to Triazophos.

Glutathione (GSH), the major non-protein thiol of cells can be easily oxidized and serve as a sink for free radicals and other reactive species. Protective and adaptive roles of GSH against oxidative stress-induced toxicity are well established in aquatic animals [38]. In the present study, a significant (ANOVA, $P < 0.05$) decrease in brain and muscle GSH level was observed. This decrease after pesticide exposure probably represents an adaptation following pesticide exposure. Previous researchers have also reported the significant ($P < 0.05$) concentration and time dependent decrease in the level of GSH [39, 40].

Increased generation of reactive oxygen species (ROS) is implicated in the pathogenesis of many diseases and in the toxicity of a wide range of compounds [41]. One of the major terminal products of LPO is malondialdehyde (MDA) [42, 43] and LPO has been a major contributor to the loss of cell function under oxidative stress [44]. As we know that brain cells have relatively low antioxidant defenses [45], hence in this study, despite of the enhancement in tissue enzymatic and non enzymatic antioxidants, increased LPO was found in the exposed zebrafish. Also, in the earlier experiment increased LPO is reported on exposure to synthetic pyrethroid, Deltamethrin [8]. Therefore, from the present result it can be inferred that the antioxidant defense in zebrafish exposed to pesticide was insufficient; free radical production is found to exceed the scavenging capability of tissue antioxidants like GSH leading to increased LPO. Also, the impairment of enzymatic antioxidant system may cause increased ROS production that may be responsible for increased LPO in both the tissues on pesticide exposure. The concentration-dependent enhancement in LPO observed in this investigation is in agreement with the other report [46]. Recently, Parathasarathy and Joseph [47] found that λ-Cyhalothrin induced a significant increase in LPO in the liver tissue as compared to control.

5. CONCLUSION

Our study revealed that neem-based pesticide Achook® induced a distinct oxidative stress in the brain and muscle of zebrafish connected with the production of ROS (reactive oxygen species). As neem trees are widely distributed in India, their formulations may prove to be an effective and eco-friendly pesticide, which could be used as an alternative of synthetic chemicals for insect control. However, an extensive research work should be undertaken on neem and its products for their better economic utilization.

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AUTHORS' CONTRIBUTION

DKS: Writing the manuscript and performed the biochemical experiments, acquisition of data, analysis and interpretation of data. BAA: Review and revision of the manuscript, administrative, technical or material support, study supervision.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Changes in caffeine content during fruit development in *Coffea canephora* P. ex. Fr. grown at different elevations

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ABSTRACT

Caffeine is an important purine alkaloid of methyl xanthine family present in beans of coffee. Caffeine content was determined in developing fruits of robusta coffee plant (*Coffea canephora*), that collected from plants grown at different elevations to find out the influence of altitude on caffeine content. Caffeine levels were analysed by performing High Performance Liquid Chromatography (HPLC). Caffeine content was found always more at all stages of coffee fruit ontogeny from plants grown at low elevations. The proportion of increase in caffeine content was more from the stage II (4 months) to stage III (5 months). Maximum caffeine content of 1.868 ± 0.149 g/100 g dry weight was evident in stage V seeds (9 months) of low altitude collected samples. There was a 32% reduction in caffeine content of beans from plants grown at high elevations.

Key words: Altitude; Endosperm; Coffee beans; Pericarp; Robusta.

INTRODUCTION

Coffee is a rich source of bioactive metabolites such as caffeine, trigonelline, chlorogenic acid, arabinogalactans, melanoidins along with ash, organic acids, and caffeic acid etc. [1] and their profile in beans is important and helpful to know the quality of coffee brew. Caffeine and it's allies theobromine, theophylline and paraxanthine are by far the best investigated compounds in coffee. Intake of such bioactive compounds from coffee drink may be associated with either health benefits or risks [2, 3]. Coffee consumption has been correlated to reduced risk of colon rectal cancer [4], type 2 diabetes [5] and Alzheimer's disease [6]. Particularly over drinking of coffee has been associated with the development of several endocrine-related cancers [7]. Caffeine ($C_8H_{10}N_4O_2$) is a bitter, white crystalline xanthine alkaloid [8] and a stimulant that keeps us awake and its systematic name is 1,3,7-trimethylxanthine (Fig. 1).

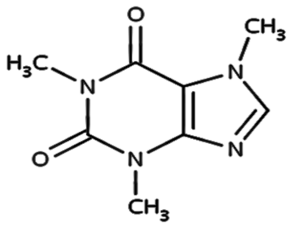

Figure 1. Structure of caffeine.

Recent studies demonstrated caffeine's positive effects such as psychoactive response viz., neurological condition such as Parkinson disease infant hyperactivity, and metabolic disorder like

diabetics gallstones and liver function [9]. The demand for high quality coffee is rapidly increased over the last few years. Biotechnological intervention for improving the quality of coffee by regulation of caffeine production through genetic engineering has been reviewed [10, 11]. Though arabica coffee brewed from beans of *Coffea arabica* is preferred as filter coffee, robusta coffee from *C. canephora* too is having importance in global market as it is used as soluble coffee (instant coffee). The levels of caffeine vary among *Coffea* spp., viz., ~1% in *C. arabica*, up to 2.5% in *C. canephora*, 1.2% in *C. dewevrei* and 1.4% in *C. liberica* [12], which is quite less than that of 4.7% of Guarana and 3.5% of tea leaves [13]. The altitude at which coffee is grown plays a major role in determining the quality of the bean. Arabica beans grow at altitudes between 600 and 1,800 meters above sea level and take six to nine months to mature. Robusta plants grow at low altitudes (sea level to 600 meters). They are more cold and moisture-tolerant and disease-resistant than Arabica. In India, though both arabica and robusta are under cultivation, robusta coffee is often blended with arabica. In the present study investigations were made to analyse the changes in caffeine content in beans of *Coffea canephora* (robusta) that harvested from plants grown at different altitudes.

2. MATERIALS AND METHODS

2.1. Samples collection

Fruits of *C. canephora* P. ex Fr. CxR variety were collected from plantations grown at different elevations near Mudigere of Chikamagalur District of Karnataka during February 2009. Ripened fruits from plants grown at high (3700 ft MSL), medium (3300 ft MSL) and low altitudes (3000 ft MSL) were collected from different places viz., Devara-mane, Guthi) and Javali respectively (Lat 13° 7' 60 N, Long 75° 37' 60 E). At least ten random plants were selected at each of the said altitude, and at least 250 g of fresh fruits were collected from these plants. These samples were immediately taken into sterile polythene bags with small perforations and used for experiment within 24 hours. To determine caffeine contents beans from fruits of five different stages were harvested i.e. Stage I (3 months DAF), Stage II (5 months DAF), Stage III (7 months DAF), Stage IV (8 months DAF) and Stage V (9 months DAF). These stages roughly represent pinhead stage immediately after dormancy period, rapid expansion stage, pericarp growth stage, efficient endosperm formation stage and lastly dry matter accumulation stage to harvesting stage respectively.

2.2. Extraction and HPLC analysis

Fruit pulp was removed and green beans were taken out. The seeds were allowed to dry to attain ~12-15% moisture content. All the green beans were then grounded finely. To determine caffeine 40-50 mg of this powder was used for alkaloid extraction and the tissue was crushed using 80% ethanol using mortar and pestle and resultant slurry was homogenized using neutralized sand the extract was centrifuged for 10 min at 8000 rpm and can be improved supernatant collected after centrifugation. The extract was flash evaporated to dryness and dissolved to 1ml of 80% ethanol prior to estimation of caffeine metabolites by HPLC [14]. HPLC analysis was performed on Shimadzu LC 20 A (Shimadzu Corp., Kyoto, Japan) equipped with CLASS-VP integrator software for data processing. The size of the column used was C-18, pore size 110A^{o}, particle size 5.0 µm, length 250 mm and internal diameter 4.60 mm (Gemini column of Phenomenex, USA). HPLC separation using UV reversed phase was performed at ambient temperature applying isocratic mobile phase consisting of methanol/ water (25:75 v/v) and flow rate was 0.8 ml/min. The mobile phase was degassed by vacuum filtration through a 0.22 µm filter and the detector wavelength was set at 270 nm. The compounds were identified by their retention times, chromatographic comparisons with authentic caffeine standard (Sigma-Aldrich, USA), and their UV spectra. Quantification was based on the external standard method.

Five coffee bean samples, out of the ten samples each that were randomly collected from coffee plants grown at higher, medium and lower elevations were extracted and analysed for caffeine. The samples were extracted and analysed in five replicates. Values from all five replicate determi-

nations of each sample were averaged and represented as means with standard deviations. Data were analysed statistically by the SPSS 17.0 software by Two-way ANOVA and homogenous subsets were determined to separate the mean values of the different altitudes and developmental stages. Means with statistical significant difference (different subsets) was marked with different alphabetical letters. Linear regression was calculated for caffeine, with elevation as the independent variable.

3. RESULTS

There were significant variations in caffeine levels of green beans of fruits collected from plants grown at different altitudes. The caffeine levels were positively influenced by altitude variation, with 18.5, 1.5 and 1.25 mg/g dry wt. bean at low, medium and high altitude samples respectively (Figure 2). Similarly there was significant variation observed in caffeine levels during the ontogeny of coffee bean development (Figure 2).

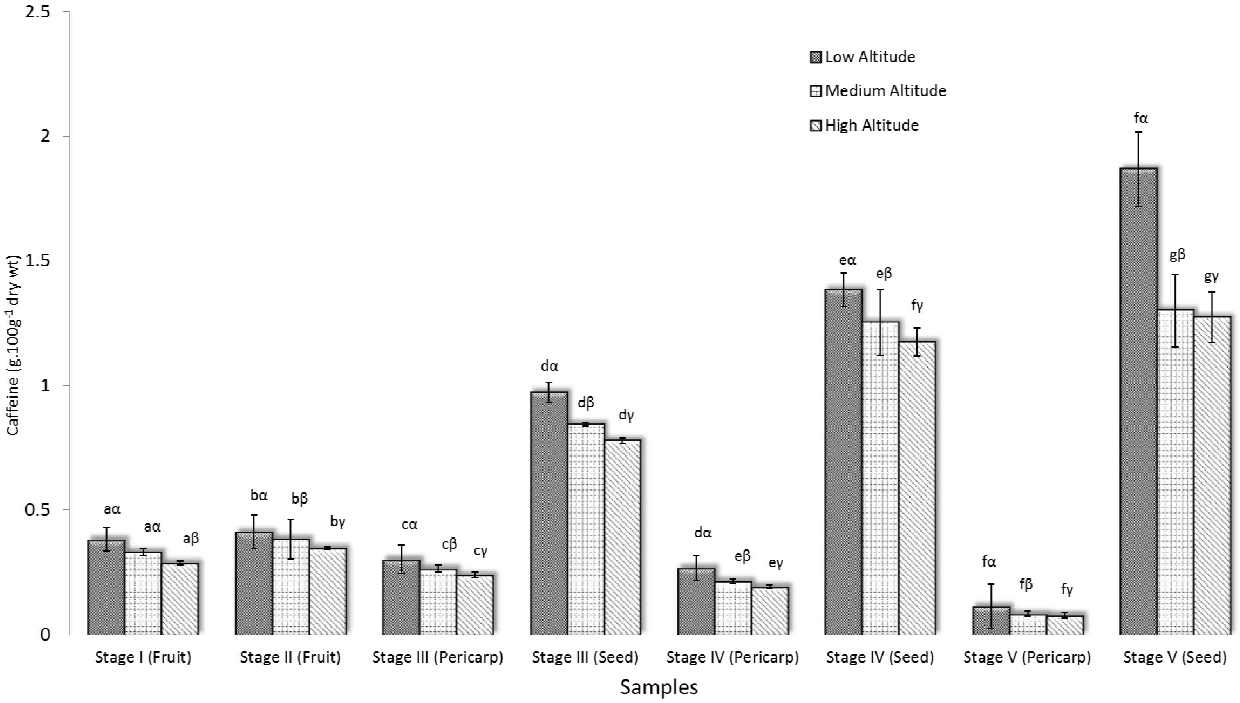

Figure 2. Caffeine profiles in beans of *C.canephoara* CxR variety during ontogeny of fruit (values are mean ± SD of five analyses), Different alphabet letters indicate the statistical significant difference within the different developmental stages ($p < 0.05$), and different symbols (α, β and γ) represent statistical significant difference within the altitude ($p < 0.05$).

In stage I samples (3 months DAF) i.e. immediately after dormant period, wherein very small fruits appear, the caffeine content was 0.381 ± 0.047 to 0.287 ± 0.009 g/100 g dry wt. Maximum content of caffeine was detected in harvested beans (Stage V, 9 M DAF). The proportion of increase in caffeine content was more from the stage II (5M DAF) to stage III (7M DAF). In seeds of stage III fruits, there was 14% and 20% increase in caffeine content in samples from medium and high altitudes compared to low altitude samples. A progressive increase in caffeine content of stage V seeds was evident from low altitude collected samples (1.868 ± 0.149 g/100 g dry wt) and there was 32% increase in high altitudes samples. To validate the efficiency of extraction procedure one sample was extracted six times and the coefficient of variation was 1.95%. Similarly a linear relationship between caffeine concentration and UV absorbance (270 nm) was perceived, wherein the linearity was managed over the concentration range of 10-100 ppm and the correlation coefficient for the caffeine standard curve was 0.9999. The retention time of caffeine was 13.56 min (Figure 3) with a relative standard deviation of RSD = 0.54%. The method of detection was 0.030 ppm and the precision was

1.95% at 50 ppm caffeine concentration. The spiked recoveries of caffeine were 1.02% for tested coffee bean extract. The variation in retention time for caffeine of test samples was insignificant. Caffeine concentration at advanced stages of fruit development decreased significantly with increasing elevation (Figure 2). The coefficient of correlation (R^2) was recorded as 0.5938 at stage V beans (Figure 4a). Similarly the average caffeine content of all stages also decreased and inversely proportional to elevation, wherein the coefficient of correlation (R^2) was recorded as 0.8553 (Figure 4b).

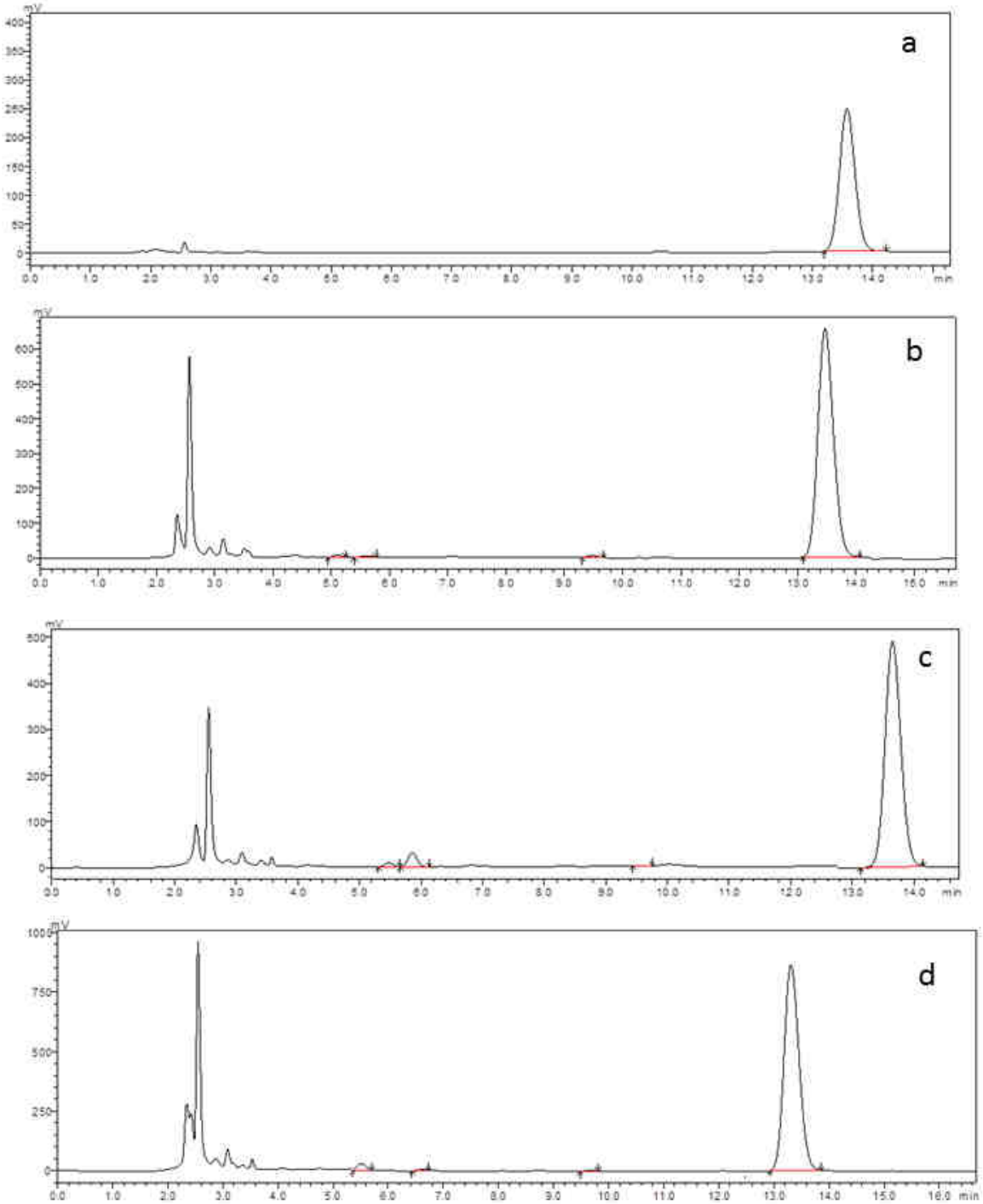

Figure 3. HPLC chromatogram of caffeine (a) standard (RT 13.56 min), (b) samples from low altitude (RT 13.49 min), (c) samples from medium altitude (RT 13.58 min), (d) samples from high altitude (RT 13.47 min).

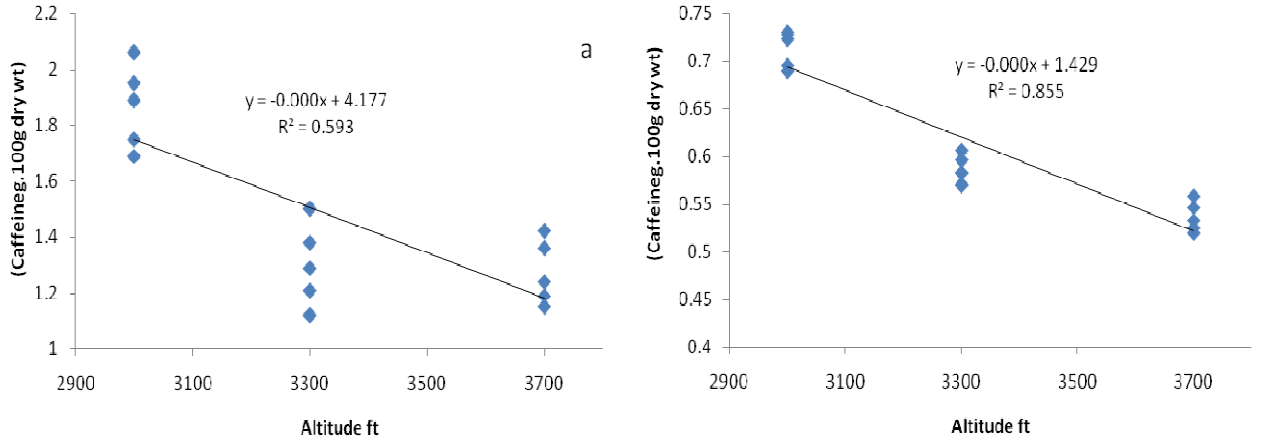

Figure 4. Regression coefficients for effect of elevation on (a) variation in caffeine content in stage V (9 months DAF) beans of *C. canephora* fruit, (b) variation in average caffeine content during ontogeny of *C. canephora* fruit.

4. DISCUSSION

In the present study, there was significant variation in caffeine content in developing seeds during ontogeny of *C. canephora* fruits. A decreasing trend in caffeine content of pericarp was evident when the fruit becomes mature and the rate of reduction was more (58%) between stage IV to stage V fruits. The trend was same in samples collected from plants grown at different altitudes. In *C. canephora* in general the fruit development requires 8-9 months or even up to 10 months and to some extent it is asynchronous and could be responsible for variation in major secondary metabolites in beans during ontogeny of coffee fruit. However, a tendency for synchrony was observed during the later stages of maturation when a significantly high proportion of fruits entered the largest sized ripe 'cherry' stage as opined [15]. Caffeine was well documented from over 63 plant species world wide [16, 17, 18] and also from different *Coffea* species [19]. Caffeine is normally synthesized in almost all parts of coffee plant, especially in young tissues such as leaves, even in floral parts [20] and highly concentrated initially in pericarp [20]. Our results too indicate that though caffeine was found in significant amounts in pericarp during initial growth stages of fruit, its accumulation was less in beans at maturation stage of fruit, which was supported by earlier studies.

The high levels of caffeine in harvesting stage beans (Stage V) is mainly due to acquisition from the perisperm, import from the pericarp [21] and also due to high activity of S-adenosine methionine (SAM) dependent N-methyl transferases involved in caffeine biosynthesis in developing endosperm [22, 23]. Apart from this metabolism aspects of caffeine in developing fruits of *Coffea*, catabolism of caffeine too influence the caffeine content of beans. The ratio between biosynthesis and biodegradation controls the difference in caffeine levels during ontogeny especially at fruit ripening stage as reported in *C. arabica* and *C. dewevrei* [24]. The altitude at which coffee is grown plays a major role in determining the quality of the bean, because there is less oxygen, coffees grown at high altitudes take longer to mature than plants grown at low altitudes. This allows the flavours to develop more fully and produces beans that are delicate and flavourful. High-grown coffee beans usually have a high density than low-grown beans. Longer maturation times and increased bean sizes are usually observed for plants grown at high altitude or in shade conditions [25, 26].

In *C. arabica* the effects of shade on the development and sugar metabolism were investigated and also showed that shade grown plants produce less caffeine [27, 28]. Similarly, post-harvest processing conditions and brewing methods too influence the metabolic profile of beans [29]. Though caffeine production takes place in pericarp of young fruits, the same will decline in pericarp of ripened fruits [30]. Similarly the caffeine content in seeds during fruit development was varied and there was a progression in caffeine content with the advancement of age of fruit. The increase in caffeine content was more significant from stage III to V. Though caffeine biosynthesis actively takes

place during leaf let emergence [31], also the same takes place in developing fruit, the pericarp and perisperm [32] reported that, absolute amounts of caffeine parallel the dry weight of bean, with more or less constant caffeine content during maturation, nearing 1% on a dry matter basis at the time of harvest.

The increase in caffeine content during stage III (6 months) to stage IV (8 months) in our study is further supported by similar observations in *C. arabica* [31]. The levels of caffeine in developing seeds are important though we don't have any information about the actual role of this in developing fruits unlike polyamines and free diterpenes [33, 34]. Most of the caffeine that synthesized in pericarp shifts to endosperm of seed where, it accumulates apart from its own caffeine content, and once further caffeine synthesis stops in pericarp in ripened fruits, caffeine content rather stabilizes in matured seeds of harvested fruits [21]. This study indicates that the altitudes at which coffee plants grow had influence on caffeine content.

AUTHORS' CONTRIBUTION

Conception and design: PG and VS; Development of methodology: PG; Acquisition of data: VS; Analysis and interpretation of data, writing, review and/or revision of the manuscript, administrative, technical or material support: VS and PG; Study supervision: PG. All authors are involved in drafting the manuscript, read and approved the final manuscript.

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CASE REPORT

First report of *Curvularia malucans* causing severe leaf necrosis of *Curculigo orchoides* in India

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ABSTRACT

Black Musale (*Curculigo orchioides*) is one of the most important *Rasayana* drug mentioned in Indian traditional system of medicine. In the recent times, many novel chemical compounds have been isolated and characterized from this medicinal plant species. This medicinal plant species is under the threat of extinction and requires *ex situ* and *in situ* conservation. During September 2013, *Curculigo orchioides* grown in the area of Guwahati, India was found to be affected with severe necrotic leaf spots. Infected leaves were collected and brought to the Forest Protection laboratory of Rain forest Research Institute, Jorhat, India for diagnosis. The leaf samples were surface sterilized and cultured on potato dextrose agar. Monoconidial isolates grown at 28°C showed brown to blackish brown mycelium with a black reverse and abundant sporulation. Symptoms were first described and the causal organism was identified as *Curvularia maculans.* This is the first report of *C. maculans* associated with a severe leaf necrotic disease of *C. orchoides* from India. Since Black Musale is an important source of novel compounds having high medicinal value, better understanding of this disease is relevant in order to establish suitable disease management strategies.

Key words: *Curculigo orchioides*; *Curvularia*; Medicinal plant; Black Musale.

1. INTRODUCTION

Black Musale (*Curculigo orchioides*) is one the highly useful plants in the indigenous system of medicine. It is a small perennial herb, up to 30 cm high with tuberous root stock bearing black fleshy lateral roots. The plant is occurring wild in subtropical Himalayas from Kumaon eastwards, ascending up to 1830 m in Khasi hills, Manipur and the Eastern Ghats. It is known by various vernacular names such as: Talmuli, Talusa (Bengali); Nelatigade (Kannada); Nelappana (Malayalam); Nilappanai (Tamil); Nelatadi (Telgue); Talmuli, Musikaparni, Talpatrika (Sanskrit); and Golden Eye Grass in English.

Pharmacological studies showed that *C. orchioides* is having adaptogenic, anti-inflammatory, anticancer, anticonvulsant, sedative, antidiabetic androgenic and immune-promoting activities. The plant is a potent source of mucilage, phenolic glycosides, saponins, aliphatic compounds and also considered as a key component of diverse herbal preparations of the Chinese and Kampoo medicine [1]. The roots are extensively utilized in pharmaceutical industries for manufacturing medicines and considerable amount of roots are extracted by the local inhabitants and tribal people from forest [2]. Curculigoside, a phenolic glycoside is the major bioactive compound of *C. orchoides* can be developed as a new drug for the treatment of

Alzheimer's disease in the future [3]. Due to over exploitation, destructive mode of collection, and other biotic and abiotic factors, this important plant species is facing genetic erosion and are under the threat of extinction. This plant species requires *ex situ* and *in situ* conservation due to its immense importance in drug discovery. Keeping the above facts in mind we conducted a survey to identify the causes causing reduction in the yield of *C. orchiodes* biomass. We observed a severe leaf necrotic disease causing total crop failure at Guwahati, India. The symptoms were first described and the causal organism was identified as *C. maculans.* Further, the pathogencity was proved in glass house condition using standard method. This is the first report of *C. malucans* associated with a severe leaf necrosis of *C. orchoides* in India and worldwide**.**

2. CASE PRESENTATION

During the mid September of 2013, a new disease (> 80% disease incidence) was observed on *Curculigo orchoides* in a nursery raised by North Eastern Development Finance Corporation (NEDFi) near Guwahati, India. The disease was found to be responsible for reducing *C. orchiodes* bio-mass substantially and constitute a potential threat to this important medicinal plant species. Symptoms on the leaves appear as small narrow, dark color lesions. As these lesions develop, the classic symptoms was observed as long, irregular black colored necrotic lesions that form parallel to leaf margins. Multiple lesions may form on a leaf, and lesions can coalesce to form large, irregular areas of dead tissue that could involve large portions on the centre of the leaves and on the margins (Figure 1). Sections from symptomatic leaves were surface- disinfested for 1 min in 70% ethanol and plated on potato dextrose agar (PDA) at 28°C. Isolations consistently yielded a fungus that was grown in pure culture (Figure 2). Conidia were brown, barrel shaped, 3 septate, broadest in the middle, the two middle cells concoloros with each other and darker than the apical and basal cell. The apical and the basal cell with broadly rounded outline. Conidiophores were brown, simple, unbranched, erect, straight or bent, septate, up to 5 µm broad of variable length (Figure 3). Morphological

Figure 1. Leaf necrosis symptoms on *Curculigo orchoides* caused by *Curvularia malucans*.

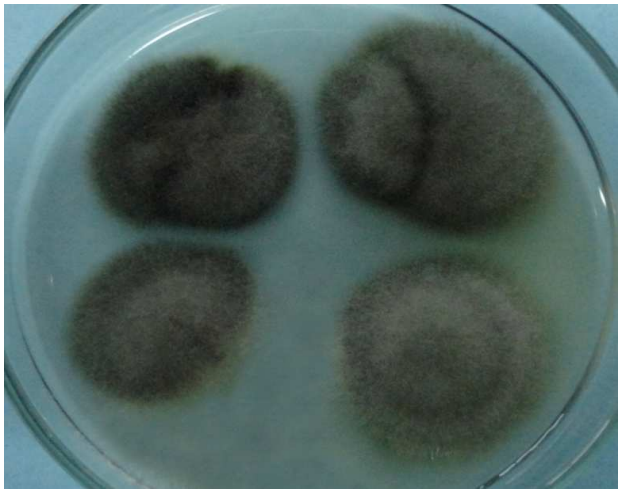

Figure 2. Pure culture of *Curvularia maculans.*

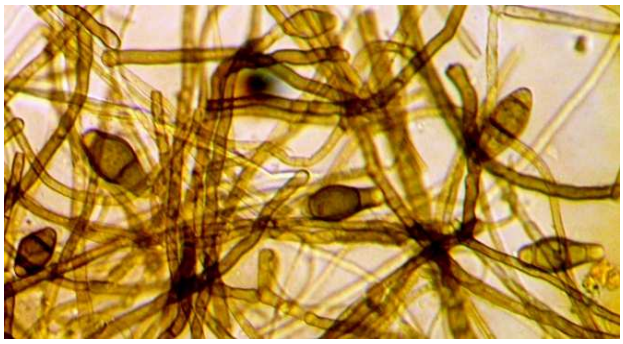

Figure 3. Conidia and conidiophore of *Curvularia malucans.*

characters agree well with the description given for *Curvularia maculans* [4]. The association of the isolated fungi with the host was further confirmed by challenge icoculation experiment. To conduct pathogenecity test, plants were grown under glass house conditions. Conidial suspensions were pre-

pared from 21-day-old cultures and adjusted at a concentration of 10^5 conidia/ ml using a haemocytometer. Suspension was sprayed onto young leaves of healthy plants. Control plants were sprayed with sterilized distilled water. Inoculated and control plants were covered with plastic bags for 72 h in a glasshouse at 28 ± 2°C. Necrotic spots of the leaves were observed after three weeks from which *C. malucans* was successfully re-isolated thus fulfilling Koch's postulates. Controls remained symptomless. Review of the pertinent literature revealed that different species *of Curvularia* are known to cause leaf spot diseases in India and China [5, 6]. *C. maculans* have been documented as causal agents of leaf spot in oil palm [7]. However, to the best of our knowledge, this is the first record of *Curvularia malucans* associated with a severe leaf necrosis disease of *C. orchoides* in India and worldwide.

3. CONCLUSION

Curculigo orchoides is an endangered medicinal plant and required to be conserved and domesticated. The leaf necrotic disease was found to reduce the yield of this economically important plant species. Further studies are needed on the ecology and spread of *Curvularia malucans* to formulate steps for the effective management.

AUTHORS' CONTRIBUTION

Conception and design: SP; Development of methodology: SP, GM and KG; Acquisition of data: RK, RR; Analysis and interpretation of data writing, review and/or revision of the manuscript, administrative, technical or material support: SP; Study supervision: SP. All authors are involved in drafting the manuscript, read and approved the final manuscript.

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ORIGINAL ARTICLE

Two new records to the flora of the Arabian Peninsula from Yemen

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ABSTRACT

The Arabian Peninsula contains a rich and varied flora, which is still incompletely known. In the period 2008-2011 have been conducted field studies and collections in the area of the Toor Al-Baha district, Lahej province, Republic of Yemen. The result of these studies was recorded in the first time to the flora of the Arabian Peninsula, two species of vascular plants: *Allium subhirsutum* L. (Alliaceae) and *Justicia ladanoides* Lam. (Acanthaceae). *Allium subhirsutum* L. was found at altitude 1214 m a.s.l., where it grows in rocky place, while *Justicia ladanoides* Lam. was found at altitude 1095 m a.s.l., where it grows in rocky limestone places. Descriptions, habitats, distributions and figures of these new records are given.

Key words: *Allium*; *Justicia*; New records; Vascular plants; Arabian Peninsula; Yemen.

INTRODUCTION

The Republic of Yemen lies in the south-western corner of the Arabian Peninsula. It extends between latitudes 12° 40’ to 19° 00’ N, and longitudes 42° 30’ to 53° 05’ E. It is bordered by Kingdom of Saudi Arabia in the north, the Arabian Sea and the Gulf of Aden in the south, Sultanate of Oman in the east, and the Red Sea in the west. It comprises about 527.970 sq. km. The study area (Toor Al-Baha) is a district of Lahej governorate. Lahej governorate is located at the south-western part of Yemen at latitudes 12° 30’ and 14° 00’ N, and longitudes between 43° 30’ and 45° 30’ E. This governorate is bounded by Abyan governorate on the east, by Taiz governorate on the west, by Al-Bayda, Al-Dhalaa governorates and some parts of Taiz governorate on the north, by Aden governorate and the Gulf of Aden on the south. The central city of Lahej governorate (Al-Hawtah) distances from Sana'a (the capital of Yemen) about 320 km south (Fig. 1).

Figure 1. Map of Yemen (source: www.google.com).

The flora of Yemen is very rich and diverse. Species diversity is a result of considerable climatic changes in former periods, which enabled different species to survive, in the different ecological habitats [1]. Former studies have reported that there are about 2838 plant species belong to 1068 genera and 179 families in Yemen [2, 3]. Since considering the southwestern part of the Arabian Peninsula as a part of the hotspots of the world [4- 7], a high attention has been paid to study of the vegetation and flora of the Arabian Peninsula particularly the southwestern part including Yemen.

In recent time there were more than 200 new records of vascular plants added to Yemen flora [8-11]. In fact, Toor Al-Baha district was rarely visited by botanists until the last decade of the 20th century. This area was already known as a rich site in succulents and is the type locality of some species [12-15]. In this paper the author collected two interesting species belonging to the genus *Allium* and *Justicia* (Toor Al-Baha district, Lahej governorate, Yemen). The specimens collected were compared with the relevant data in the literature [3, 16-20].

As a result of all these comparisons, the specimens collected were found to be new records for the Arabian Peninsula flora from Yemen.

2. MATERIALS AND METHODS

During the intensive floristic survey between 2008 and 2011, the author was collected plant samples from different habitats of Toor Al-Baha district, Lahej governorate, southern Yemen. The specimens were first compared with similar species from flora of Yemen and then flora of Kingdom of Saudi Arabia, Flora of Oman, flora of Somalia and flora of Ethiopia, in order to identify them. The collected specimens were pressed and mounted on herbarium sheet and deposited in the herbarium of Biology Department, Faculty of Education, Aden University (Yemen). The references [18-21] were used for the identification of the taxa.

3. RESULTS

During the present study two species viz., *Allium subhirsutum* L. (Alliaceae) and *Justicia ladanoides* Lam. (Acanthaceae), were recorded for the first time for Yemen, and were not previously recorded for the Arabian Peninsula.

A list of the species arranged alphabetically within their families is given with citations of their description, synonym, type, habitats and distribution.

1. *Allium subhirsutum* L. Sp. Pl. 1: 295. 1753. (Fig. 2)

Synonyms: *A. spathaceum* Steud. ex A. Rich. (1851); *A. subhirsutum* var. *spathaceum* (Steud. ex A. Rich.) Regel (1875).

Type: Linn 4193.

Herb smelling mildly of garlic when crushed; bulb globose to ovoid-oblong, to 1.5 cm diameter, bulb coat membranous, grayish. Leaves (1-)2-3, linear, flat or slightly keeled when fresh, 8-50 x 0.2-2 cm, hairy at least along margins; sheaths 1.5-14 cm, mostly below ground, pale white or yellow, glabrous or hairy especially near the top. Inflorescence-stem single (sometimes 2 or 3), solid, uniform in thickness, shorter or longer than leaves. Spathe opening along1 slit, persistent, up to 1.3 cm long, with green or pale purple veins, shorter than pedicles. Inflorescence an umbel or spherical cluster, 2-7 cm in diameter, few- to many-flowered. Pedicles to 10-40 mm long, bracteoles absent. Flowers campanulate to stellate; tepals spreading white with a pale-red midvein, elliptic or oblong, 5-8.5 mm long, the outer slightly wider than the inner, obtuse or acute, joined together and also to the filaments for c. 1 mm at the base; filaments ½ to ⅔ the length of the tepals, filaments simple; pistil shorter or longer than perianth, ovary globose to obovoid with style attached about half way above the base; style 3-6 mm long, slender with 3-1obed stigma. Capsules subglobose, 3-6 mm in diameter. Seeds black c. 2.5-3.5 mm long.

Habitat: The specimen was collected from a single locality in Jabal Athumah, Toor Al-Baha district, Lahej province, where it was found growing in rocky place at alt. 1214 m a.s.l.

Phytochory: Mediterranean

Previous report from Yemen: None.

Specimens examined: Yemen, Toor Al-Baha district, Lahej governorate, on alt. 1214 m a.s.l., 13° 02' 505" N, 44° 16' 111" E, 10. 4. 2010, Othman 4229.

Figure 2. *Allium subhirsutum* inflorescence in habitat. Photography taken by the author.

2. *Justicia ladanoides* Lam., Tabl. Encycl. 1(1): 42 (1791). (Fig. 3)

Synonyms: *Tyloglossa kotschyi* Hochst. (1843); *Justicia kotschyi* (Hochst.) Dandy (1956); *J. schimperi* (Hochst.) Dandy (1956) var. *kotschyi* (Hochst) J. K. Morton (1978).

Type: without collector and locality.

Erect or straggling annual or short-lived perennial herb 0.1-1.5 m high. Stems glabrous to densely pubescent with spreading or descending non-glandular hairs; internodes up to 5-18 cm long. Leaves: blade 2-11 x 1-5 cm, linear, narrowly ovate to ovate-elliptic or elliptic, attenuate to rounded at the base, acute to obtuse or acuminate at the apex, lateral veins 5-7 pairs, sparsely to densely pubescent with ascending appressed non glandular hairs; petiole in distinct up to 5-40 mm long, pubescent with spreading non-glandular white hairs. Flowers several together at the upper nodes. Bracts 6-12 x 1.5-3 mm, linear to narrowly obovate or narrowly elliptic, ciliate along the margins. Bracteoles 1.5-3.5 x 0.3-0.6 mm, linear to subulate. Calyx lobes 4-6 x 1-1.2 mm, subulate, with green middle and white hyaline margins, nearly glabrous except for the margins which are ciliate or fimbriate, or sparsely pubescent with mostly ascending non-glandular hairs and also rarely with glandular hairs. Corolla purple or very rarely white with a white/purple pattern on lower lip near throat, 17-20 mm long; tube 8-10 mm long, 2-4 mm wide at the mouth, pubescent outside; lower lip 8-10 mm long with the 3 lobes 2.5-3 x 2.3 3 mm; upper lip 4-6 mm long and bifid at the apex. Stamens with filaments 4-6 mm long, glabrous; anthers usually yellow or occasionally purplish 1.2-2.4 mm long, lower theca with a 0.3-0.6 mm long tail. Ovary 1.8-2 mm long, glabrous; style 11-14 mm long, pubescent below half; stigma with subequal lobes. Capsule 7-10 x 2.5-3 mm, sterile basal portion 2.5-3 mm long, acute to obtuse at the apex, glabrous or rarely sparsely pubescent at apex. All capsule 4-seeded; seeds 1.5-2 x 1.1-1.5 mm, triangular-discoid, flattened, densely verrucose.

Figure 3. *Justicia ladanoides*, A: habit, B & C: fruiting branches**.** Photographs taken by the author.

Habitat: It was collected from a single locality in Jabal Athumah, Toor Al-Baha district, Lahej province, where it grows in rocky limestone places at alt. 1095 m a.s.l.

Phytochory: Sudano-Zambezian

Previous report from Yemen: None.

Specimens examined: Yemen, Toor Al-Baha district, Lahej governorate, on alt. 1095 m a.s.l., 13° 02' 736" N, 44° 16' 078" E, 22. 3. 2010, Othman 4110.

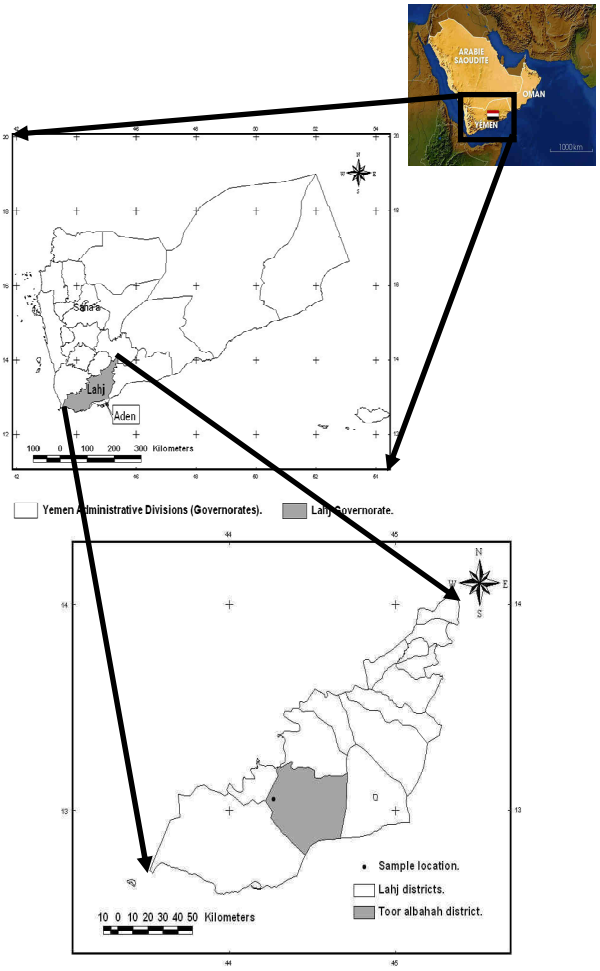

Figure 4. Location map of Yemen, Showing its administrative divisions (governorates) browsing location of Lahej governorate of which the study area is within (at Toor Al-Baha district) where *Allium subhirsutum* and *Justicia ladanoides* were found.

4. DISCUSSION

The *Allium* specimens collected from Toor Al-Baha district, Lahej province, Yemen, were first compared with the related species in floras of the neighboring countries. It was seen that the specimens were markedly different from the related *Allium alibile* Steud. ex A. Rich. due to the fact that the specimens collected from Toor Al-Baha district were leaves hairy at least along margins and the anthers are shorter than the perianth [18]. The morphological characteristics of the species were identical to those of *Allium subhirsutum*, which is distribution in N Sudan, N Somalia, Djibouti, Ethiopia and Eritrea [18, 20].

Key to closely related *Allium* species:

1. Bulb single and prominent, with or without small, spherical bulblets, plant not cultivated.
2. Umbel with a ring of scarious bracteoles; anthers longer than the perianth; leaves smooth → *A. alibile*
2. Umbel without bracteoles; anthers shorter than the perianth; leaves hairy at least along margins → *A. subhirsutum*
1. Bulb made of several more or less equal bulblets or single and virtually indistinguishable from the base of the scape; plants cultivated.

The *Justicia* specimens collected from Toor Al-Baha district, Lahej province, Yemen, were first compared with the related species in floras of the neighboring countries. According to this comparison, the specimens looking like *Justicia heterocarpa* T. Anders. due to the fact that specimens collected from Toor Al-Baha district were corolla purple, more than 10 mm long, bracts more than twice as long as wide and capsules 4-seeded. The morphological features of the collected speci-mens were identical to those of *Justicia ladanoides*. This proves the presence of this species, which is known to grow in Senegal to Ethiopia, extending north to Sudan and south-east Egypt, southwards to northern Zaire, Uganda and Kenya [19, 21].

Key to closely related *Justicia* species:

1. Plant herbaceous; capsules at least some of them, 4-seeded; seeds tuberculate, somewhat flattened.
2. Corolla purple, usually more than 10 mm long; bracts usually more than twice as long as wide; capsules smooth, 4-seeded → *J. ladanoides*
2. Corolla pink, less than 10 mm long; bracts less than twice as long as wide; capsules with 6 deeply dissected wings and 1-seeded → *J. heterocarpa*
1. Plant herbaceous; capsules 2-seeded; seeds smooth, strongly flattened.

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The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Influence of methoprene on the larval biochemistry in haemolymph and fat body of *Ephestia cautella* Walker (Lepidoptera: Pyralidae)

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ABSTRACT

The almond moth, *Ephestia cautella* Walker is a serious pest of stored cereals, nuts, and dried fruits. The haemolymph and fat body play major roles in the insect developmental physiology. Proteins and amino acids are important biochemical constituents of haemolymph and fat body of the insects. Methoprene (a juvenile hormone analogue), that specifically disrupts the normal development of insect. Changes in the total protein and total free amino acids level were studied in the haemolymph and fat body of third instar larvae of almond moth exposed to sublethal concentrations of methoprene. The sublethal concentrations of methoprene i.e. 1, 2 and 4 ppm caused a significantly dose-dependent reduction in the level of total protein and an associated enhancement in the total free amino acids level in haemolymph and fat body tissues of the *E. cautella* larvae. The maximum decrease in total protein level in haemolymph (45% of the control value) and fat body (52% of the control value) was observed in larvae treated with 4 ppm of methoprene while the maximum enhancement in the total free amino acids level in haemolymph (167% of the control value) and fat body (148% of the control value) was also observed in larvae treated with 4 ppm concentration of methoprene. These effects on overall physiology of the almond moth leading to depletion of the protein contents cause several shortages for normal development and reproduction.

Key words: Methoprene; Haemolymph; Fat body; Total protein; Total free amino acids.

INTRODUCTION

The almond moth, *Ephestia cautella* Walker is a serious pest of stored cereals, nuts, dried fruits and cereal commodities. Preventive measures utilizing conventional grain protectants (organic insecticides) may result in the presence of chemical residues in foodstuffs that may eventually be destined for human consumption. There is an evidence of increasing incidence of resistance to conventional insecticides among the major insect pests of stored products. In addition, their persistent use affects immune system of insects and of course pollutes our own environment due to non-biodegradability, biomagnifications and toxicity to non-target organisms. In such condition safer substitutes are the need of the present environment. Methoprene (a juvenile hormone analogue, JHA) is a highly effective, sesquiterpenoid hormone that regulates growth and development in insects. Since, JHA signaling is specific to insects and other arthropods, compounds disrupting JH action in

insects are an ideal target for pest management, due to their low toxicity to nontarget organisms outside the Arthropoda. In insect fat body is a dynamic tissue involved in multiple metabolic functions. One of these functions is to store and release energy in response to the energy demands of the insect [1]. Fat body cells not only control the synthesis and utilization of energy reserves - fat and glycogen, but also synthesize most of the haemolymph proteins and circulating metabolites. Large amounts of relevant proteins, such as storage proteins (used as an amino acid reservoir for morphogenesis), lipophorins (responsible for the lipid transport in circulation), or vitellogenins (for egg maturation), are synthesized and secreted by the fat body [2]. To perform multiple metabolic functions to fulfill the changing physiological needs of the insect during development, the fat body must be able to integrate signals from other organs. Many of these functions are hormonally regulated, and thus the fat body is the target organ of several hormones [3]. The fat body coordinates insect growth with metamorphosis or reproduction by storing or releasing components central to these events [4].

Haemolymph is the only extra cellular fluid in the insect body that is usually kept in circulation by an open heart within the body cavity. It makes up 15-75% of the volume of the insect. It transports food materials and hormones to the cells and metabolic waste products away from those same cells. The blood cells or haemocytes lie suspended in plasma and serve principally in phagocytosis, i.e. the ingestion of small solid particles [5]. They also ingest bacteria and the tissue fragments which result from histolysis during pupation. Hormones that regulate larval moulting, growth, longevity, metamorphosis, metabolism and reproductive behavior of insects are secreted and circulated in the haemolymph [6]. One of the most characteristic features of insect haemolymph plasma is the high level of free amino acids ranging from 25 to 75 mM, and functioning as buffer in osmoregulation and as substrates for protein synthesis, energy production for flight and cocoon construction whereas insect fat body is an active site for the transamination between amino acids [7].

Juvenile hormone analogues have been shown to affect the level of total protein and total free amino acids in haemolymph and fat body of insects [8, 9].

In the present study, initial biochemical analysis has focused on compounds such as protein and amino acids level in almond moth larvae, exposing different sublethal concentrations of methoprene. The effects of JHAs on metabolic homeostasis and energy metabolism in insects are poorly understood. The most common effect of JHA treatment is the disruption in the levels of hemolymph and fat body metabolites. The metabolic effects of JHAs may also be manifested as a result of the morphogenetic effects of the compound on the insect.

Hence, as an objective of such programme the experiments were designed and conducted to investigate the effect of methoprene on the total protein and total free amino acids level in haemolymph and fat body of the third instar larvae of *E. cautella*. The present investigation besides exposing the insecticidal influence on the larval biochemistry of this pest would also provide measures for its efficient chemical control.

2. MATERIALS AND METHODS

A rich standard culture of the almond moth, *Ephestia cautella* Walker was maintained in the laboratory on a normal dietary medium composed of coarsely ground wheat (*Triticum aestevum*) mixed with 5% (w/w) yeast powder and 10% (w/w) glucose inside large glass containers (150 mm diameter, 200 mm height) at a temperature of 26 ± 1^0C, relative humidity 93 ± 5% and a light regime of 12 hours light and 12 hours darkness. Methoprene was used throughout the investigation and was obtained from AccuStandard, Inc.125 Market Street, New Haven, CT 06513. For the preparation of different concentrations of methoprene in dietary media, a stock solution of known concentration of methoprene was prepared in required volume of acetone and then adjusted via serial dilutions to achieve its required concentrations. Now required volume of different concentrations of methoprene were thoroughly mixed with the required quantity of normal food (coarsely ground wheat, *Triticum aestevum* mixed with 5% (w/w) yeast powder and 10% (w/w) glucose) to get desired concentrations of methoprene. This treated

food was then air dried at room temperature to eliminate completely the excess of the organic solvent. For control purposes, the normal food was thoroughly mixed with a required volume of acetone similar to that of treated food and then air dried in the same way. For biochemical estimations, only three sublethal concentrations i.e. 1, 2 and 4 ppm were prepared.

From above culture, whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Eggs laid by these females were collected and then placed in glass chambers for hatching. Newly hatched larvae thus obtained were allowed to feed on a normal dietary medium (kept inside 250 ml beakers) for 20 days. On the 21st day, 25 third instar larvae were transferred to each similar rearing chamber containing dietary medium mixed with above mentioned concentrations of methoprene, and were allowed to feed for 10 days. 25 larvae were kept as control with each set of experiment. On the completion of 30 days, 10-15 larvae from each set, experimental as well as control were taken out. From these groups of larvae, haemolymph and fat body were separately collected and pooled in a manner outlined as thus.

Haemolymph was obtained from these larvae following the procedure of Krishna and Pandey [10] which involved making of a small puncture by means of a sharp needle at the dorsolateral side of the prothoracic segment and drawing the blood, easily oozing out through this puncture, into a fine glass capillary tube. The haemolymph thus obtained from caterpillars was collected in a previously weighed small glass vial (12 mm diameter; 55 mm height). For each biochemical estimation, after ascertaining the weight of the haemolymph, known volume of required solvent was added to prepare the homogenate.

Fat bodies were taken out from these larvae following careful dissections performed on a clean glass slide containing minute quantities of distilled water under a stereoscopic binocular microscope. The water and the flowed out haemolymph surrounding these tissues were then completely drained off with the help of absorbant paper. Later, this fat body was weighed and swiftly mixed with known volume of required solvent to prepare the homogenate for each biochemical estimation.

The total protein was measured according to the method of Lowry et al. [11] using bovine serum albumin as standard. The absorbency was measured at 600 nm against blank prepared simultaneously by using 1.0 ml of 1 N NaOH instead of 1.0 ml of tissue extract. The absorbency was compared with that of varying concentrations of bovine serum albumin (20 mg/ml to 200 mg/ml). Appropriate calculations were made to compute the total protein content of the tissues. Results were expressed as µg protein/mg wet weight of tissues.

Estimation of total free amino acids was carried out according to the method of Spies [12] using glycine solution as standard. The absorbency was measured with the help of spectrophotometer, systronics Digital Type 106 (MK II) at 575 nm against blank prepared in the similar way by using 0.1 ml of 96% ethanol instead of 0.1 ml of supernatant. The absorbency was compared with that of varying concentrations of glycine solution (10 µg/ml to 100 µg/ml) in double distilled water. Appropriate calculations were made to compute the total free amino acid levels. Results were expressed as µg free amino acid/mg wet weight of tissues.

Results have been expressed as the mean ± s.e. of six replicates. Significant differences between treatment groups, in order to show dose-dependence, were determined by one way analysis of variance [13].

3. RESULTS

Changes induced by sublethal concentrations of methoprene (1, 2 and 4 ppm) on the levels of total protein and total free amino acids in haemolymph and fat body tissues of the larva of *E. cautella* have been represented in Table 1. Methoprene caused a significantly dose- dependent reduction in the level of total protein and an associated enhancement in the total free amino acids level in both the tissues of the larva.

In case of control larval groups, the total protein content in the haemolymph and fat body was 54.97 and 67.89 µg/mg respectively. The maximum decrease in total protein level in haemolymph (45% of the control value) and fat body (52% of the control value) was observed in larvae treated with 4 ppm concentration of methoprene. Total protein levels, in haemolymph,

were reduced to 88 (48.76 µg/mg), 68 (37.47 µg/mg) and 45% (24.79 µg/mg) of the control while these levels in fat body were reduced to 89 (61.08 µg/mg), 73 (49.73 µg/mg) and 52% (35.49 µg/mg) of the control following treatment with 1, 2 and 4 ppm concentrations of methoprene respectively. The total free amino acids content, in the control larvae, was 68.56 and 12.61 µg/mg in haemolymph and fat body respectively. The maximum enhancement in the total free amino acids level in haemolymph (167% of the control value) and fat body (148% of the control value) was observed in larvae treated with 4 ppm concentration of methoprene. Total free amino acid levels, in haemolymph, were increased to 113 (78.16 µg/mg), 125 (86.13 µg/mg) and 167% (114.85 µg/mg) of the control while these levels, in fat body, were increased to 118 (14.98 µg/mg), 130 (16.47 µg/mg) and 148% (18.76 µg/mg) of the control following treatment with 1, 2 and 4 ppm concentrations of methoprene respectively. We also found morphological abnormalities in larvae such as abnormal mouth parts of larvae.

Table 1. Changes in the total protein and total free amino acids level in the haemolymph and fat body tissues of the larva of *Ephestia cautella* treated with methoprene

Methoprene concentration (ppm)	Total protein [#] (µg/ mg, wet wt.)		Total free amino acids [#] (µg/ mg, wet wt.)	
	Haemolymph	Fat body	Haemolymph	Fat body
Control (Untreated)	54.97 ± 1.36 (100)	67.89 ± 1.44 (100)	68.56 ± 1.46 (100)	12.61 ± 0.45 (100)
1	48.76 ± 1.16 (88)	61.08 ± 1.99 (89)	78.17 ± 1.08 (113)	14.98 ± 0.23 (118)
2	37.47 ± 1.19 (68)	49.73 ± 1.76 (73)	86.13 ± 1.47 (125)	16.47 ± 0.61 (130)
4	24.78 ± 0.66 (45)	35.49 ± 1.22 (52)	114.85 ± 1.29 (167)	18.76 ± 0.63 (148)

[#] Values have been expressed as the mean ± s.e. of six replicates.
Values in the parentheses indicate the percentage change with control values taken as 100%.
Analysis of variance showed that the response to the methoprene was dose-dependent $p < 0.001$.

4. DISCUSSION

Proteins are among the most complex of all known chemical compounds of living organism. They serve as an important internal environmental factor for the metabolism, especially having a close relation with fat body, metamorphic hormone, trehalose and sex hormone during development and metamorphosis. Protein are synthesize in the early instars of the larval fat body (the main site of protein synthesis) and subsequently released into the surrounding blood [14], which, in later instars are sequestered from the blood into the fat body. Regarding their synthesis, Simmon and Mitchell [15] have suggested that in *Drosophila* amino acids are first incorporated into peptides and later enter into proteins [16].

In the present investigation, all the three sublethal concentrations of methoprene caused a dose-dependent ($p < 0.001$) reduction in the level of total protein in both the tissues of the larva. Similarly, Zibaee et al. [17] has reported that application of pyriproxifen significantly depleted protein level in haemolymph and fat body of the *Eurygaster integriceps* Puton. Bosquet and Calvez [18] have also reported that treatment of *Bombyx mori* larvae with methoprene caused a significant reduction in the hemolymph proteins. Mulye and Gordon [19] observed that LD_{50} concentrations of the juvenile hormone analogue (methoprene) caused decrease in protein concentrations in the hemolymph of sixth instar larvae of *Choristoneura fumiferana*. Similarly, Etebari et al. [9] suggested that application of pyriproxifen residue significantly decreased the total protein in the hemolymph of the larvae of silk worm. Bosquet et al. [20] reported that the JHA treatment decreased major hemolymph protein synthesis without any accumulation of untranslated mRNA, suggesting that methoprene treatment affected translation of mRNA for protein

synthesis. Alternatively, the lower than normal haemolymph protein levels may be the consequence of increased utilization and uptake by other tissues rather than reduced production by the fat body. Methoprene caused a dose-dependent ($p < 0.001$) enhancement in the level of total free amino acids in both the tissues of the larva of *E. cautella* (Table 1). Similarly, Zeenath and Nair [21] found that when the sixth instar larvae of *Spodoptera mauritia* were treated with juvenile hormone analogue (hydroprene), the total free amino acids concentration increased. Recently, at 14 ppm concentration effects of methoprene at larval stage was as much severe that this concentration caused 100% larval mortality in *E. cautella* [22].

Since, methoprene in the present study, decreased the protein level in the haemolymph and the fat body of the larva of this moth as stated earlier [19-21], it may be concluded that a rise in the total free amino acids level in both the tissues is plausibly on account of protein depletion and/or inhibition of amino acids incorporation into protein. The imbalance of biochemical constituent resulted abnormalities in developmental physiology such as retention of old cuticle, lethargy, loss of body fluids. Abnormalities in mouth part leading to starvation of larvae which may be another cause for reduced protein synthesis or increased utilizing of protein in heamolymph and fat body tissues of larva of almond moth.

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AUTHORS' CONTRIBUTION

Both authors are involved in drafting the manuscript, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Photosynthesis, respiration and carotenoid contents in the green alga *Botryococcus braunii* at elevated nutrient levels

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ABSTRACT

Carotenoids content of *Botryococcus braunii* displayed low nitrate demand; the concentration of 3.2 mM nitrate (50% of Chu 13 medium) resulted in most of the growth magnitude whereas carotenoids increments were highest at 25% (1.6 mM) nitrate. P_N (net photosynthetic oxygen evolution) and carotenoids content responded opposite to each other at supplemental bicarbonate; carotenoids have been enhanced at low concentrations (0, 1 or 5 mM), being highest at 100% nitrate (6.4 mM) while P_N was significantly enhanced at higher concentrations (10, 15 and 20 mM) and *vice versa*. Enhanced P_N at high carbon and nitrogen may have shifted the metabolism to amino acid synthesis thus inhibiting hydrocarbons synthesis. NaCl (0.15 or 0.2 M) increased carotenoids content 5 times that of the control (zero NaCl) whereas higher salinity concentrations (0.25 and 0.3 M NaCl) were relatively inhibitory. Salinity, in addition, abolished the usual preferential effect of nitrate concentrations on carotenoids accumulation since their level was only slightly less at 6.4 mM than at 50% this concentration (3.2 mM). Collectively, NaCl (0.1-0.2 M) induced highest carotenoids and chlorophyll contents, highest P_N and lowest R_D in 50% nitrate whereas does not have such effect in 100% nitrate. *Botryococcus braunii* proved strong capability of adjusting the pH of the medium, whether acidic or alkaline, back close to the start. Carotenoids content was not responsive to a wide range of pH values (4-12), exhibiting their highest level at pH 6.

Key words: *Botryococcus braunii*; Carotenoids; Media components; Photosynthesis; Respiration.

INTRODUCTION

Algae for biofuel production are receiving more interest in research because of many advantages, not least for their ability to use low quality water and land: thereby do not compete with food production. Furthermore, yield estimates are significantly higher for algae than for any other crop when compared on the basis of needed land area [1]. Algae cultivated on 30 million hectares with a biofuel yield of approximately 40,000 liters per hectare and year is sufficient to replace the 1,200 billion liters of petroleum used by the United States. This area is similar to that used for soya plantations in the United States (about 29 million hectares) and roughly twice that used for the US production of corn ethanol (about 14 million hectares were used to produce almost 64 billion liters

of ethanol in 2011). *Botryococcus braunii* is a green colonial photosynthetic microalga widespread in fresh and brackish water with a global distribution in temporal and tropical oligotrophic lakes and estuaries [2, 3]. It is an important green algal species in the field of biotechnology, because of its ability to synthesize and accumulate a variety of hydrocarbons up to extraordinary levels. Depending on the strain and growth conditions, up to 70% of the algal dry mass can be in the form of hydrocarbons [4]. Blooms of the colonial green alga, *B. braunii*, are widely known to exert toxic effects on a variety of aquatic organisms and have been noted to cause fish death in some environments [5]. The hydrocarbons can be used as fuel and feedstock for the chemical industry. Hydrocarbons extracted from the alga can be converted into biodiesel and gasoline by convential catalytic cracking [6] that may be one long-term solution to reduce dependence on fossil fuels. *Botryococcus braunii* varieties are also known to produce exo-polysaccharides, with amounts being dependent on strain and culture condition [7].

Due to its outstanding capacity for lipid accumulation, *B. braunii* is continually attracting scientific interest. However, the persisting problem of slow growth rate is a barrier in obtaining large amounts of algal hydrocarbons. We, therefore, are investigating suitable culture conditions for ideal growth of this alga. Examining growth under varied carbon/nitrogen ratios representing the focus of the present study, based on our hypothesis that the accumulation of hydrocarbons requires relatively more carbon and much less nitrogen (there is no nitrogen in the carotenoids chemical structure). The hypothesis was examined by applying various nitrate concentrations and nitrate-bicarbonate combinations in the culture media. As a consequence of sodium bicarbonate addition, the pH of the medium and Na content increased. Therefore NaCl salinity was also examined to characterize the role of sodium if salinity, osmotic or ionic stresses are exerted. In addition to growth and carotenoids content, photosynthetic and respiratory activities were also considered under a series of nitrate, $NaHCO_3$, NaCl, their combinations and a wide range of pH values.

2. MATERIALS AND METHODS

Botryococcus braunii (SAG 807-1) was kindly offered by (Sammulung Von Algenkulturen Gottingen, Germany). Cells of *Botryococcus braunii* were cultivated under sterile conditions in cultivation room on 1.5% agar plates with modified Chu 13 medium [8] modified by Largeau et al. [9] and kept at a temperature 25±2ºC for stock culture maintenance. Experimental culture for control and standard growth conditions were cultivated in 250 ml conical flasks containing 150 ml of liquid Chu 13 medium at 25ºC/23ºC (day/night) at PAR 20 µmole.m^{-2}.s^{-1} provided by white fluorescents lamps and shaken. The following modifications have been applied to the medium for treatment cultures:

1. Different levels of potassium nitrate (0.0, 1.6, 3.2, 4.8 and 6.4 mM) as 0, 25, 50, 75, and 100% relative to the original concentration in Chu 13.
2. Different levels of $NaHCO_3$ (0, 1, 5, 10, 15 and 20 mM).
3. Different levels of NaCl (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.3 M) in combination with 3.2 or 6.4 mM nitrate (50% or 100%)
4. Different pH values of 2, 4, 6, 7, 8, 9, 10, 12 (adjusted by H_2SO_4 and NaOH) in combination with 0.15 and 0.2 M NaCl.

The following analytical methods were conducted at 15 days old cells: Absorbance at 750 nm was followed daily throughout the experimental period (15 days). Chlorophylls (a and b) and carotenoids content were determined in methanolic extracts according to Holden [10]. The net photosynthetic oxygen evolution (P_N) and dark respiratory oxygen uptake (R_D) were monitored using a Clark type electrode computerized to an Oxygen Monitoring System (OMS, Hansatech instruments Inc, donation from the Alexander von Humboldt Foundation Germany to R. Abdel Basset). A volume of 2 ml was followed under continuous filtered (red) light intensity of 100 µmole.m^{-2}.sec^{-1} at 25°C for 30 min and the rate was calculated as 20 µmole O_2.mg Chl^{-1}.min^{-1}. The same sample was also measured but in the dark for respiratory oxygen uptake. Chlorophyll content of analogous sample was estimated and calculated as before. The pH

values were measured using a pH meter (HANNA Instruments Inc Woonsocket-RT-USA, Romania). All the experiments have been repeated several times and the mean values of three replicates ± standard error are presented in the graphs.

3. RESULTS

The green algae *Botryococcus braunii* was grown in Chu 13 medium with a large range of carbon and nitrogen (C/N ratio) targeting the highest growth and/or carotenoid content. The series of experiments started with examining the effect of nitrate concentrations on growth determined as O.D. 750_{nm} (not shown), and as content of chlorophyll a and b (Chl a and b) as well as carotenoids. The chlorophyll content increased (Fig. 1) as the concentration of nitrate was increased up to 100% relative to the content of Chu 13 medium (6.4 mM KNO_3). However, the most noticeable increase in chlorophyll (32.6% relative to the initial content) occurred at 3.2 mM nitrate where as doubling nitrate concentration to 6.4 M induced only about 77% higher amount of chlorophyll. Carotenoids, on the other side, exhibited their major increase at a much lower concentration of nitrate (1.6 mM), leveled off at around 3.2 mM while have been negatively affected by higher concentrations.

In the next set of experiments elevated C/N ratios were analyzed by supplementing the cultures with various concentrations of $NaHCO_3$ (0, 1, 5, 10, 15 or 20 mM final) in combination with different levels of nitrate (0-6.4mM). As a consequence of the addition of bicarbonate the pH of the medium raised; concentrations of bicarbonate higher than 1mM (5-20 mM) resulted in a pH close to 10 (data not shown). After 15 days of growth at both 1 and 5 mM bicarbonate, cells of *B. braunii* were able to lower the pH of the medium back closeto the initial pH values. Nitrate concentrations of 1.6, 3.2 and 6.4 mM almost doubled the chlorophyll content whereas nitrate deprivation severely lowered chlorophylls with no significant impact of increased bicarbonate concentrations (Fig. 2). However, carotenoid contents varied widely depending on the level and combination of bicarbonate and nitrate (Fig. 3).

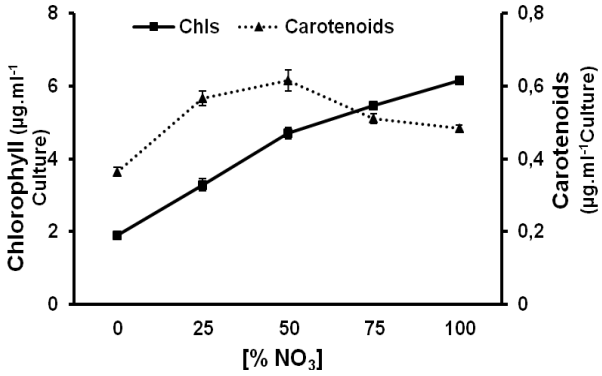

Fig. 1. Chlorophyll and carotenoid contents of *B. braunii* as influenced by successively increasing concentrations of nitrate. The mean values of three replicates ± SE are presented in the graph.

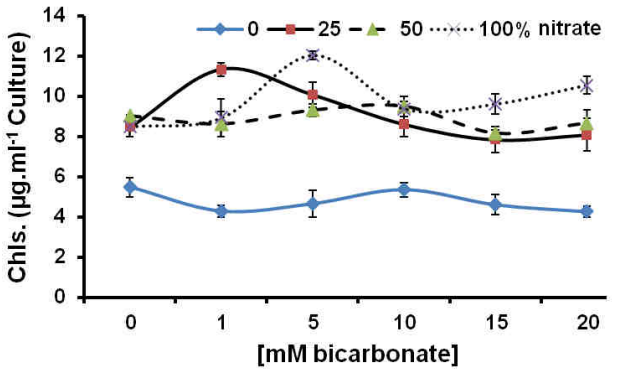

Fig. 2. Chlorophyll contents of *B. braunii* as influenced by successively increasing concentrations of sodium bicarbonate combined with different nitrate concentration 0, 1.6, 3.2 and 6.4 mM, shown on the graph as 0, 25, 50 and 100% relative to that in Chu 13 medium. The mean values of three replicates ± SE are presented in the graph.

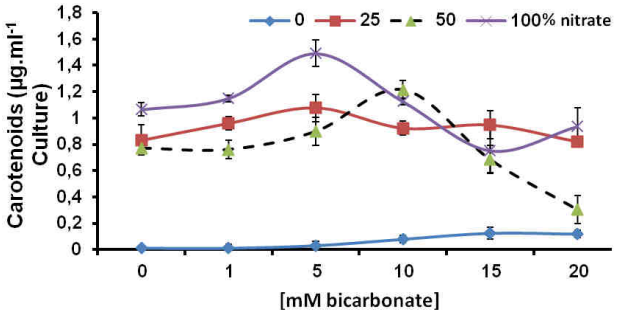

Fig. 3. Carotenoids contents of *B. braunii* as influenced by successively increasing concentrations of sodium bicarbonate; nitrate levels as in Fig. 2. The mean values of three replicates ± SE are presented in the graph.

Addition of 0.1, 1.0 or 5.0 mM bicarbonate markedly enhanced the accumulation of carotenoids, the highest contents being recorded at combi-

nation of 5 mM bicarbonate with 6.4 mM nitrate; followed by 3.2 mM nitrate. Both 15 and 20 mM bicarbonate decreased the level of carotenoids at all concentrations of nitrate. No impact of bicarbonate was observed at 25% nitrate. At zero nitrate, carotenoids were of small values but started to appear when bicarbonate concentrations become above 5 mM.

Photosynthetic oxygen evolution (P_N) of *B. braunii* has also been markedly influenced by the applied carbonate and nitrate combinations (Fig. 4). The concentrations of 10, 15 and 20 mM bicarbonate enhanced P_N to its highest rates in the presence of 1.6 and 3.2 mM nitrate while relatively lowered at highest nitrate of 6.4 mM. The drop in O_2 yield at 20 mM $NaHCO_3$ could be a pH effect. The lowest bicarbonate concentrations (0, 1 and 5 mM) did not show any effect on P_N of *B. braunii*. Full inhibition of photosynthesis was almost observed at all bicarbonate concentrations examined when nitrate was totally deprived. Respiratory oxygen uptake (R_D) responded to the applied treatments quite opposite to P_N. The highest rates were recorded at zero nitrate while enhanced at increased bicarbonate concentrations (Fig. 5). Respiratory rates were almost similar at 1.6, 3.2 and 6.4 mM nitrate but decreased as bicarbonate concentrations were increased.

The addition of $NaHCO_3$ increased sodium concentration in the culture media, thus imposing a salinity impact on the cells. Therefore, *Botryococcus braunii* was grown in successively increasing NaCl concentrations. The notion that most of the growth enhancement resulted from 3.2 mM nitrate followed by that at 6.4 mM (50% and 100% of Chu 13 medium, respectively), led to conduct the forthcoming experiments i.e. successively increasing salinity levels in the presence of either of these concentrations. Inclusion of moderate salinity levels (0.1-0.2 M NaCl) significantly enhanced chlorophylls (a and b) contents at 3.2 mM nitrate but not at 6.4 mM. Highest levels of NaCl (0.25 M and 0.3 M NaCl) were severely suppressive to chlorophylls at both nitrate levels. Chl. b, same as $O.D._{750nm}$, was gradually decreasing as NaCl level was increased (data not shown). Carotenoid contents responded quite different from chlorophylls.

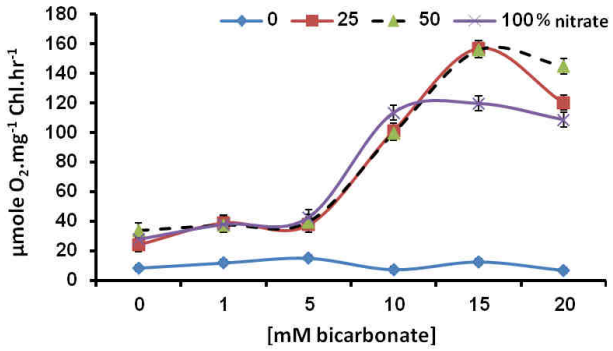

Fig. 4. Photosynthetic oxygen evolution of *B. braunii* as influenced by successively increasing concentrations of sodium bicarbonate; nitrate levels as in Fig. 2. The mean values of three replicates ± SE are presented in the graph.

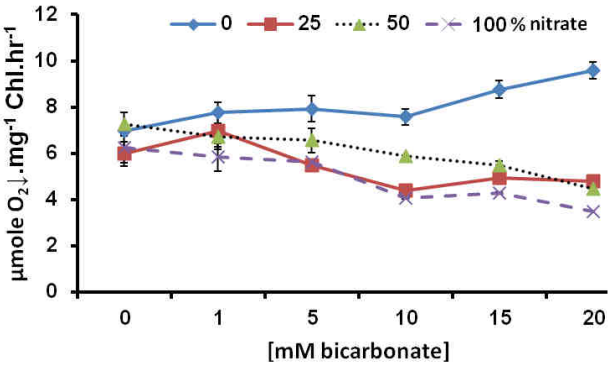

Fig. 5. Respiratory oxygen uptake of *B. braunii* as influenced by successively increasing concentrations of sodium bicarbonate; nitrate levels as in Fig. 2.The mean values of three replicates ± SE are presented in the graph.

They increased by the very low concentration of sodium chloride (0.05 M) and up to 0.2 M NaCl. Carotenoid contents increased 5 times at 0.15 or 0.2 M NaCl relative to that of the control (zero NaCl) while higher salinity concentrations of 0.25 and 0.3M NaCl were inhibitory to carotenoids accumulation. However, the inhibitory effect of high salinities on carotenoids was less than that on chlorophyll a. Also, no pronounced difference between salinity (0.15 or 0.2 M NaCl) in either of the two nitrate levels (50 and 100%) concerning carotenoid contents. Aging was significantly stimulatory to carotenoid accumulation, almost doubled within the last three days from day 12 to day 15 (not shown).

Photosynthetic oxygen evolution (P_N) of *B. braunii*, grown at 100% nitrate (6.4 mM) was not affected by salinity. Cutting nitrate concentration to 50% more than doubled the P_N in salinities

up to 0.1 M NaCl; higher salinities gradually inhibited P_N. Respiration of *B. braunii* at 6.4 mM increased steadily by increasing salinity level and by aging (not shown). At half nitrate level (3.2 mM), however, respiration exhibited lowest rates at a salinity range from 0.1 to 0.2 M NaCl; otherwise it was higher. Such response of respiration to NaCl and nitrate combinations is greatly opposite to that of photosynthesis. Putting these together, NaCl (0.1-0.2M) with 50% nitrate induced highest chlorophyll contents, highest P_N and lowest R_D.

The pH of the medium has been elevated as a result of sodium bicarbonate inclusion. Therefore, the most stimulatory concentrations of nitrate (3.2 mM) and NaCl (0.15 M or 0.2 M NaCl) were combined and studied at abroad range of pH values to define which pH is the optimum for growth and carotenoid accumulation. Figure 7 shows obvious ability of *B. braunii* to adjust the pH of the medium in either case, acidic or alkaline. Highest modulation of pH was observed in cultures started at pH 4 or 12 as cells of *Botryococcus* adjusted the pH to about 8 or 9; respectively. However, at extreme acidity (pH 2) such ability has not been displayed by the alga, in either of the salinity levels. Cultures containing either 0.15 or 0.2 M NaCl exhibited identical capability of adjusting the pH of the medium; for simplicity only that at 0.15 M NaCl is presented in Figure 7. Growth as O.D. 750nm (not shown) and chlorophyll (a and b) were, almost doubled at 0.2 M relative to 0.15 M NaCl; maximally at pH 9-10 (Fig. 8). Furthermore, higher salinity of 0.2 enabled the alga to be more tolerable to alkaline media. The carotenoids content, however, was clearly different from that of growth and chlorophylls, being of their highest levels at pH 6 (Fig. 9). Carotenoids were continually increasing as the cells become older up to 11 days (except at pH 8 and 10 they were of higher values at younger ages). Because they were quite similar in effect and level, only those at 0.15 M NaCl are presented. Lowest pH of 2 was severely depressing to carotenoid contents at both levels of NaCl. P_N was not markedly affected by a wide range of pH values (4-10) but severely inhibited at extreme pHs of 2 and 12 (Fig. 10).

Elevating salinity level to 0.2 M NaCl, suppressed half of the P_N activity at 0.15 M, irrespective to the pH at which *B. braunii* was grown and this was the single difference between the two salinity levels (Fig. 10). Respiratory oxygen uptake in the dark (R_D) of *B. braunii* exhibited minor dependence on the pH of the medium (not shown).

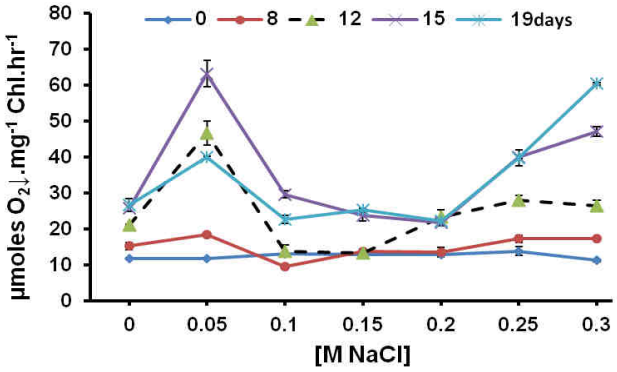

Fig. 6. Respiratory oxygen uptake of *B. braunii* as influenced by successively increasing concentrations of sodium chloride combined with 3.2 mM nitrate (50%). The mean values of three replicates ± SE are presented in the graph.

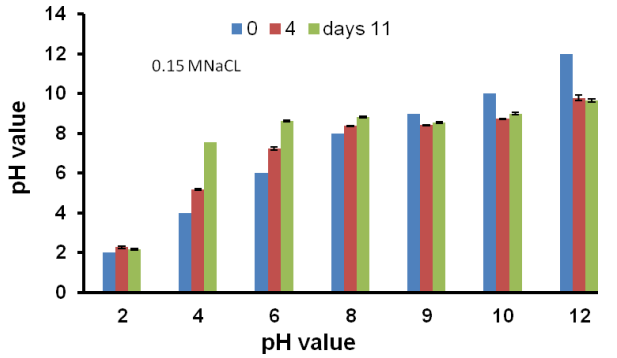

Fig. 7. Initial (immediate to addition of sodium bicarbonate) and final pH values as modified by *B. braunii* after growth for 15 days. The mean values of three replicates ± SE are presented in the graph.

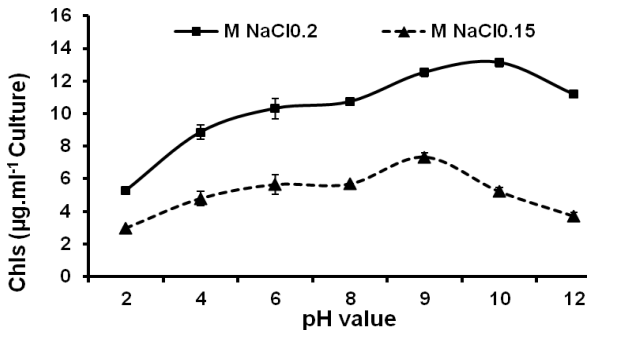

Fig. 8. Chlorophyll contents of *B. braunii* grown for 15 days at various pH values in the presence of 0.15 or 0.2 M NaCl. The mean values of three replicates ± SE are presented in the graph.

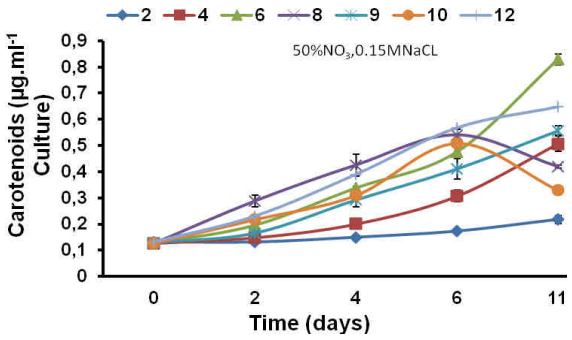

Fig. 9. Carotenoid contents of *B. braunii* grown for 15 days at various pH values in the presence of 0.15 M NaCl. The mean values of three replicates ± standard error are presented in the graph.

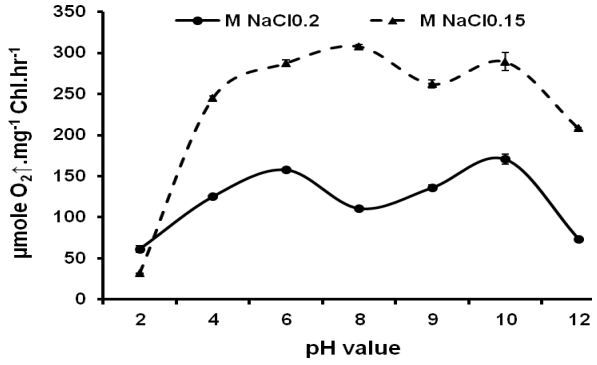

Fig. 10. Photosynthetic oxygen evolution of *B. braunii* grown for 15 days at various pH values in the presence of 0.15 or 0.2 M NaCl and 3.2 mM nitratel. The mean values of three replicates ± standard error are presented in the graph.

4. DISCUSSION

The outstanding capacity of accumulating hydrocarbons up to characteristic levels has drawn a special attention to *Botryococcus braunii*. The different metabolic pathways and the microbial engineering for the production of advanced biofuels have been recently reviewed by Peralta-Yahya et al. [11]. Yet, the slow growth rate, along with low growth level, is irremediable for feasibility of mass hydrocarbons production by *Botryococcus braunii*. Metzger and Largeau [12], and Ergluet al. [13] reported that very high proportions of hydrocarbon content (e.g. 86%) may be observed during the very late stationary phase and may be the result of cell lysis and loss of biomass. Dayananda et al. [14] studied optimization of media constituents for growth and hydrocarbon production. This work, as well, has been assigned to define nutrient limiting factors for growth enhancement and hydrocarbons accumulation. The low nitrate demand for growth and carotenoids of *B. braunii* is an obvious conclusion deduced from the results of this work. Cutting nitrate to 50% (relative to 6.4 mM contained in Chu 13 medium) did not impair growth of *Botryococcus braunii*. Furthermore, highest carotenoids content has been resulting from lowest nitrate level given to the cells (1.6 mM). Not only nitrate concentration could be reduced but also it can be replaced by nitrite; 50% nitrite (2 mM) was as efficient as 100% nitrate (4 mM) as reported by Yang et al. [15]. It may be concluded, then, that low nitrate is stimulatory to hydrocarbon accumulation in *B. braunii*, assuming that carotenoids are markers of hydrocarbons. These findings match our hypothesis that since nitrogen is not a constitutive element of hydrocarbons which is a great proportion of the algal biomass, cells' demand of this element must be limited. Hydrocarbons may account to 70% of the dry mass of *Botryococcus* (Abdel-Basset and Melis, Unpublished).

Further dampening down of nitrate proportion was studied by adding a series of bicarbonate concentrations to elevate the carbon/nitrogen ratios in the culture media. Bicarbonate concentrations (10-20 mM) highly enhanced P_N while relatively lower concentrations (up to 5 mM) did not. *Vice versa* responded the carotenoid contents i.e. they were enhanced by lower bicarbonate and inhibited by higher concentrations. In this respect, Ambati et al. [16] reported relative enhancement of carotenoid contents in *Botryococcus braunii* under conditions of NaCl and $NaHCO_3$. In this work, higher bicarbonate concentrations led to markedly enhanced photosynthesis that is, in turn, known to enhance nitrate reduction into ammonia; the latter is combined with photosynthesis into amino acids. Hence, carbon skeletons left for hydrocarbon synthesis may drop which interprets low carotenoids at enhanced P_N by high C/N ratios. The pathway for amino acids should be preferable for the cells, since carotenoids need much more energy (hydrogen), as they are much more reduced in comparison. Nitrate deprivation, in the presence of bicarbonate, inhibited P_N and carotenoids while enhanced R_D. In this respect, carbon and nitrogen interrelationship has been reported to affect hydrocarbons content. Rao et al. [17] reported that CO_2

at 2.0% (v/v) caused a two-fold increase in biomass and carotenoid contents in all the *B. braunii* strains studied compared with control cultures (without CO_2 supplementation). In the presence of glucose under light or dark conditions, the intracellular oil droplets were markedly larger than those cultured without glucose [18]. Miao and Wu [19] reported that heterotrophic growth of *Chlorella protothecoides* results in a high level of lipid accumulation.

The addition of bicarbonate in the growth media imposed an increase in sodium and pH, simultaneously. Sodium chloride was studied to characterize the effect of sodium toxicity or salinity apart from that of bicarbonate on growth and carotenoids. Carotenoid contents have been increased by the very low concentration of NaCl (0.05 M) and up to 0.2 M, increased 5 times at 0.15 or 0.2 M NaCl relative to that of the control (zero NaCl). Higher salinity levels were only slightly and relatively inhibitory to carotenoids accumulation, might be due to decreased number of cells surviving such high salinities. Meanwhile, the inhibitory effect of high salinities on carotenoids was much less compared with that on chlorophylls. Collectively, NaCl (0.1-0.2 M) in 3.2 mM nitrate-containing media induced highest carotenoids and chlorophyll a contents, highest P_N and lowest R_D. In salinized cultures, carotenoid contents were not differentially responsive to doubling nitrate concentration as usual, may be because intracellular nitrate concentrations are equalized and become no longer a limiting factor in the two nitrate levels (3.2 and 6.4 mM). This would have been mediated by a Na^+-stimulated and energy-dependent nitrate uptake system, analogous to what has been reported in *Synechocystis* sp. strain PCC 6803 [20].

B. braunii exhibited a potential ability to adjust the pH towards the initial of the growth medium, acidic or alkaline pH (at both levels of salinity). At all pHs, NaCl (0.2 M) induced higher growth magnitudes (chlorophyll contents) but less P_N and R_D. Such higher NaCl concentration (0.2 M) aided the organism's tolerance to survive extreme alkalinity (pH 10 and 12) more than the acidic ones (2 and 4), in terms of doubled chlorophyll contents compared with those at 0.15 M NaCl. Carotenoids exhibited their highest levels at pH 6. In this regard, solution pH was found to have a significant effect on the activity, aggregation size and thermal stability of PSI [21].

AUTHORS' CONTRIBUTION

AFH: Conception and design, development of methodology, acquisition of data, analysis and interpretation of data, responsible of publishing the manuscript; RA-B: material support, writing and revision of the manuscript. Authors are involved in drafting the manuscript, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Land use planning for strategic management (Case study: Kiyan protected area, Nahavand, Iran)

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ABSTRACT

History has shown that power, influence, and cultural life of civilization societies during their evolution influence by existence of forests and natural reserves. Conservation and management of these resources are essential in Iran which consider as one of the most arid regions in the world beside their good potential of biodiversity. The study area (Kiyan forest protected area) located in 12 kilometers southwest of the Nahavand city. This study, tried to show land use planning with ecological models and finally, using SWOT model guidelines proposed for strategic management in this region and another similar climate conditions. Results of land use planning of Kiyan forest reserves as a homogeneous management zone showed that the best option is protection (National Natural Monument) and the second option is Tourism (recreation outdoor). In other word, National Natural Monument, Tourism, Forestry and Range management are the main options respectively. Therefore, it is recommended that this region converted to National Natural Monument in term of management level.

Key words: Land use planning; SWOT model; Strategic Management; Kiyan forest reserve.

INTRODUCTION

Power, influence, and cultural life of civilization societies during their evolution are the main parameters which affected by existence of forests and natural reserves. Environmental and recreational resources influenced by growth of population and over exploitation from natural resources [1]. So experts declare that welfare is equal to value of forest resources divided on population [2]. In this regard, recreational land use of forests, the growing tourism industry as a source of income for improved social and economic conditions of natives; also these capabilities are principles of sustainable forest management [3]. Iran can be considered as one of the top countries in the world about natural diversity, presence of four full seasons and attractions of Islamic Iranian culture [4]. Management programs of natural resource should be done with proper division using natural values and ecological potential to achieve sustainable development [5]. There are several methods to determine ecological potential; For example, for tourism feasibility SWOT method (Strength Weakness Opportunities Threats) can be used [6]. Kiyani et al. used matrix analysis for investigation of land use and land cover changes in Taleghan area [7]. Strategic analysis is an important step in the planning process [8]. In this study, ecological models with SWOT model guidelines

applied for land use planning and strategic management in the region.

Kiyan forest is a part of Zagros forests in west of Iran in climax condition, also particular floristic composition and high species richness (405 species) are main reasons for selecting this place as a genetic protected area [9]. The flora of Kiyan region includes 64 families, 264 genera and 405 species. The life form spectrum of plant species is as follows: 7% phanerophytes, 12% geophytes, 42% hemicryptophytes, 4% chamephytes and 35% therophytes [9]. Forests of Hamadan province spread in some far-away places and divided in two categories of sparse and destroyed forest [10]. Kiyani and Khalilnejad stated that outdoor capability of Kiyan forest reserve has a suitable potential for ecotourism [11]. Kiyani and Kiyani to evaluation of Kiyan forest reserve for ecosystems management showed that because of high grazing rate and use of more than ecological carrying capacity, this capability is decreasing [12]. Shayan et al. showed existence of numerous springs and Kiyan forests, genetic reserves, have reasons for higher combination score (point of view tourism); and stated absorption of tourism will improve economic status of natives [13]. Therefore, it is necessary to keep its existence in every possible way and then restoration. Because land use planning is the process of decision-making for the management of land and resources and Land use planning plays a significant role in local government activities, this study investigate the land use planning of Kiyan forest reserve for Strategic Management.

2. MATERIALS AND METHODS

2.1. Study area

The study area (Kiyan forest protected area), known as Kiyan spring is located in south of Kiyan city and 14 kilometers from southwest of Nahavand city (Central Zagros) in Hamadan province, Iran. This area has latitude 34º 8' 21" to 34º 9' 40" North and longitude 48º 12' 55" to 48º 14' 9" east with minimum height of 1615 meters above sea level and maximum height from sea level is 2080 meters (mean height is 1850 meters above sea level). Kiyan forest reserve is the only natural forest in the Hamadan province and it is rest of Zagros forests in the west of Iran. The sequence of ecosystem succession is in the stable level (climax) and undoubtedly reaching to this level takes long time about thousands of years. However, these reserve have protected species like Oak, Walnut and Hawthorn which their natural forest area is 100 hectares and the planted and semi destructed forests is about 400 hectares [14]. Grassland vegetation cover in this area varies between 20 to 80%.

Maximum and minimum temperature of study area in summer and winter is 40 and 25°C, respectively. In geomorphological division, Naha-vand city include three physiographic sections: Alluvial Plain, Piedmont Plateau and Alluvial Fans. Kiyan area is located on alluvial fan, with deep soil and high percent of gravel [15]. In the Nahavand city because of calcareous forma-tions, rain penetrates to groundwater by dissolving the limestone and usually appears in surface as notable springs. Discharge of Kiyan spring is about 2,500 liters per second, which after drinking and farming uses of villagers, the excess water flows into the Gamasyab River. Figure 1 shows location of Kiyan forest reserve in the Nahavand Township [16].

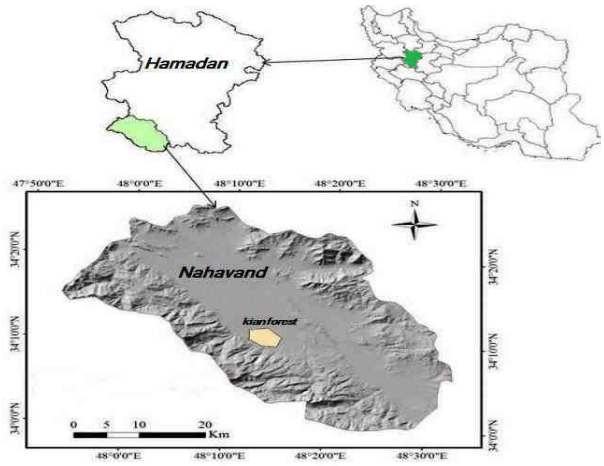

Figure 1. Position of Kiyan forest reserve in Iran [13].

Kiyan region has semi-arid climate, summers are fairly mild and winters are relatively cold. According to the regional meteorological center of Nahavand city, average annual rainfall is 360 mm (maximum is 479 mm and minimum is 221 mm); average annual temperature is about 20ºC. Water hardness of Kiyan springs is 110 mg/L $CaCO_3$ while mineral water is about 110 mg/L $CaCO_3$ [11].

Research method in this paper is survey - analytical based on field studies. Basic information of this research is based on analyze of detailed studies to identify ecological resources. The characteristics of these models are based on hydrologic, geomorphologic, climate and vegetation assessment and land classification of study area. In ecology of tourism and recreation, forestry and range management models have 3 and 7 classes, respectively [17]. Ecological models forums are general guide for assessing the ecological application. Therefore, based on hydrological characteristics, geomorphologic, climate and vegetation of area should a specific model developed [18]. Models of tourism, forestry, conservation and Range management prepared in accordance with Nahavand region and they compared with characteristics of the study area.

To identify ecological resources of Kiyan forest reserve, scientific literature reviewed. In physiographic view, Nahavand city divided to three sections: Alluvial Plain, Piedmont Plateau and Alluvial Fans. Kiyan area is located in Alluvial Fans section with deep soil and high gravel [15]. Table 1 shows characteristics of environmental units of Kiyan forest reserve. Priority indicators in evaluating tourism ecological model are: slope, stone, geography, water, vegetation, climate and weather. If the slope is not suitable for tourism area, will avoid examining of other parameters. This general rule is most true for first key indicators [17]. Important tree species include: *Cotoneaster spp., divaricata spp., Crataegus spp., Cornus australis spp., Quercus brantii, Platanus orientalis* [9].

Table 1. Environmental characteristics of Kiyan forest (Kiyani, 2013)

Altitude(m)	1600-1800
Slope (%)	More than 8 percent
Geographical aspect	Western North - Eastern North
Type of soil and rock	Sandy – loamy, semi-deep and calcareous rock
Vegetation type and density	Oak, sycamore, hawthorn, ash, hackberry, amygdalus and wild almond with density of 10-70%
Climate	Semi-arid (cold steppe to temperate alpine)
Water resource	Kiyan springs with 1.2 m^3/s
Wildlife	partridge, porcupine, jackal, wolf and goats (habitat is destructing)
Erosion risk	Risk of landslides and erosion is moderate to high

3. RESULTS AND DISCUSSION

Kiyan forest reserve has a unique natural and attractive landscape in west of Iran. Continuous use and ecotourism development will need ecological potential. After comparing features of Kiyan forest reserve with ecological model, potential of area determined is shown in Table 2.

General methods for land use planning are qualitative and quantitative methods [17]; in this study, quantitative methods used to find out priority. This method has four scenarios as below:

1- Percentage of the current land use
2- Economic needs of region
3- Social needs of region
4- Ecological needs of area

First based on above scenarios, land use prioritized and weight of 1 to 10 assigned to every land use. Then, for a deficit position or a failure of Class potential one score decrease from value weights (Table 3). Ranking is based on sum of options; finally, the best option with highest score selected for the Environment unit. The results of land use planning of Kiyan forest as a management homogeneous zone showed that best priority of options is as follows: National Natural Monument, Tourism, Forestry, Range Management, respectively.

Table 2. Assessment of ecological potential Kiyan forest reserve

Land use Unit name	**Range management (7 class)**	**Ecotourism (3 class)**	**Forestry (7 class)**	**Protected area (National Natural Monument)**
Kiyan forest reserve as a management homogeneous zone	3	1	4	Genetic value of rare species (such as oak, dangle tulip and squirrels) Source of water for agriculture Educational value Recreation Place

Table 3. Weighted values of Kiyan forest reserve

Land use scenarios	**Protected area (National Natural Monument)**	**Range Management (medicinal plants)**	**Tourism (recreation outdoor)**	**Forestry (tree farming)**
Class potential	1	3	1	4
First scenario	10	6	9	4
Second scenario	8	7	8	6
Third scenario	10	6	10	6
Forth scenario	10	5	9	5
Sum	39	27	37	25
Priority	1	3	2	4

Table 4. Matrix analysis of strategic factors in SWOT

SWOT Matrix	**Strengths**	**Weaknesses**
	1 - Natural pathway along river 2 - Moderate climate 3 - Biodiversity in the region 4 - Water supply for Kiyan city 5 - Place of recreation outdoor	1- Susceptibility to erosion and landslide 2 - High traffic of tourists especially 3 - Lack of space for picnic 4 - Lack of boundary 5 - Unsuitable entrance area
Opportunities 1 - Optimum use of rich rangelands 2 - Production of medicinal plant 3 - Fishery 4 - Increase number of guards 5 - Educational value	**SO** strategies 1 - Raise level management 2 - Construction of tell cabin 3 - Multipurpose use of rangelands 4 - Agricultural industries	**WO** strategies 1 - Buffer zones to preserve region 2 - Terracing of slopes 3 - Pay cheap loan to farmers to reduce in grazing and product of medicinal plants 4 - Replacement entrance
Threats 1 - Increase of waste materials 2 - Risk of water contamination 3 - Low tourist security 4 - loss of social networks in natural resource 5 - Not take entrance fee from native people	**ST** strategies 1 - Law enforcement of water and soil pollution from health centers 2 - Promote the culture of tourism by municipality 3 - Creation of participative management 4 - take entrance fee from all of visitors	**WT** strategies 1 - Partnership and community consultation 2 - Range Management Plan to reduce surface runoff and soil erosion 3 - Determine of ecological carrying capacity 4 - Prevent construction in region

Ecological resources of Kiyan forest reserve compared for tourism, forestry, conservation and Range management. Results indicated this region has fourth priority for forestry, third priority for Range management, second priority for recreation outdoor, and has high priority for protection. Because slope is the main factor in tourism model and slope of Kiyan spring is more than 5%, so it classified as second floor for recreation outdoor. For propose of strategic planning, results of this study were combined with previous studies; Matrix analysis of strategic factors in SWOT are presented in Table 4. Strategies are listed according to their importance.

Because about 40% of travel incentives are ecotourism and visiting natural attractions [19] Management programs of natural resource should be done with proper division using natural values and ecological potential [5] and there are several methods to determine ecological potential of a region. Strategic analysis is an important step in the planning process [8] and some studies emphasized on outdoor capability as a suitable potential for ecotourism [11]. In other hand, carrying capacity will decrease by some phenomena like high grazing rate and overuse of ecological capability [12].

Bali et al. applied Iran's ecological models in order to monitor the ecological changes in Rose plain and suggest optimal solutions of strategic factors using matrix analysis [20]. Also, matrix analysis can be used for investigation of land use and land cover changes [7]. Ebrahimpuor et al. used SWOT strategic models for tourism development planning of Lorestan province and concluded that the WO and SO strategies had the first and second priorities, respectively [21].

4. CONCLUSION

The strategic land use planning function is essentially concerned about the planned allocation of land use and the planning of movement networks. It is undertaken in a way that expresses or reflects a community's or region's vision and aspirations for its future growth and development, to be achieved over a specified period of time. A strategic land use plan can be produced at any geographic or spatial level - for a municipal district, for a metropolitan area, or a town or village, or for any specified area, especially an area experiencing significant development pressures or negative effects of growth and development.

Kiyan forest reserve is remnants Zagros forest in the west of Iran and has status of stable (climax), Particular floristic composition and high species as a genetic reservoir. Results of multi criteria evaluation method show that priority of this region from 4 to 1 belongs to forestry, Range management, recreation outdoor and protection, respectively. Therefore the best option for management of Kiyan forest reserve as a homogeneous zone is conservation (National natural monument). Based on analysis of strategic matrix, best strategy of SO was improving management level to National Natural Monument; best strategy of ST was law enforcement of Water and soil pollution by health related organizations; best strategy of WO was buffer zones to preserve the region and finally the best strategy of WT was partnership and community consultation. Finally, to sustainable development suggested that this region converted to National Natural Monument which is one of areas supported by the Environmental Protection Agency; to settle an improving condition in nature quality of region.

AUTHORS' CONTRIBUTION

NR and VK: Conception and design; NR and VK: Development of methodology; MZ: Acquisition of data; VK and MZ: Analysis and interpretation of data; NR and VK: Writing, review and/or revision of the manuscript; Administrative, technical, or material support; NR: Study supervision.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Mineralogical characteristic and geochronometry of crystalline rocks from SE part of the Lapland Granulite Belt of Kola Peninsula at the White Sea

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ABSTRACT

The Lapland Granulite Belt is placed on the Kandalaksha region at the White Sea. Research topic were granulites and amphibolites. They represent a rift zone between the Belamorian and the Kola blocks. In this article,is described the micas mineralogy from the above-mentioned rocks and granitoid veins cutting the complex. Also geochronology of these rocks on the basis of isotopic dating K-Ar and U-Pb methods were studied. Age of the zircon indicate the main stage of formation of the rocks metamorphites and rendered as a result of transformation of the protolith. Age of the micas is much younger and is already associated with subsequent processes in the area of the White Sea.

Key words: Lapland Granulite Belt; Geochronometry; Mineralogy; Metamorphic rocks; Granitoides.

INTRODUCTION

Lapland Ganulite Belt (LGB) is located in the northern part of the Baltic Shield (Figure 1). This structure separates the Belamoryan and Kola blocks and the age of the various phases of metamorphism is dated at 2.4-2.8 Ga [1-4]. The oldest rocks occurring in the LGB area are represented by granulites, exposited in the central parts of the unit, and in the Kolvitza area. In those granulites was dated a zircones. Their ages range from 2.1-3.8 billion years ago (2.6 billion by Kozlov [4]). Such a large age range of zircons are interpreted as a meta-basement origin of granulites. It is considered that these granulites are meta-morphosed sedimentary rocks which were feeding the accumulation zone, formed by abrasion of older rocks, adjacent to the reservoir, which was then LGB [5, 6]. They were formed in an island arcs, in the basin separating the Belamoryan and Kola blocks [4, 7]. The younger of granulite are meta-volcanites (usually represented by amphibolites with garnets [1, 2, 8, 9]). The age of these rocks was determined to be 2.4-2.7 billion years [4, 10, 11]). These are metamorphoied ande-sites and basalts products [4, 8, 9, 12]. Near the metavol-

canites are present the gabbroide intrusion, which have age 2,47-2,45 billion years [12]. Large intrusions of gabbro-anorthozite rocks have age 2,452±7 million [12, 13]. In the Kolvitza region, the granulite rocks and charnockites are altered by a number of processes of metamorphism. Granitoide veins rocks from Kandalaksha part of the Lapland granulite belt so far not been investigated.

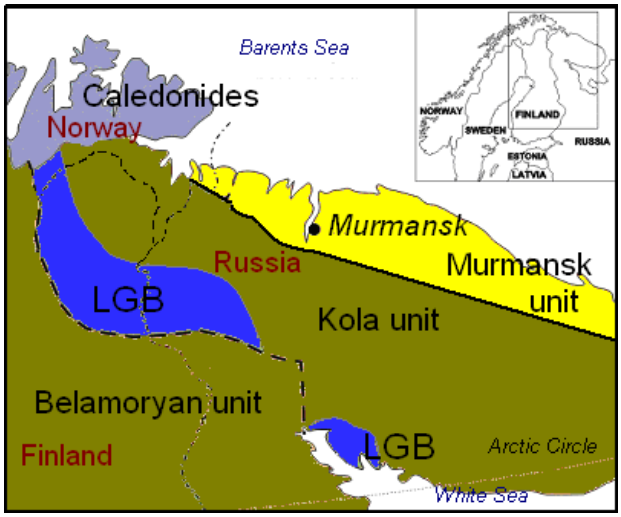

Fig. 1. Localization of Lapland Granulite Belt on the Balic Shield (based on Lauri et al. [14], Pozhilenko et al. [15] and author's studies).

2. MATERIALS AND METHODS

The collected samples of rocks from the Kandalakshan part of the Lapland Granulite Belt were analyzed using an optical microscope Leica DM2500P and scanning electron microscope Hitachi SU6600 with EDS which are located on the Optical and Electron Microscopy Laboratory in Department of Geology and Lithosphere Protection, UMCS. The geochronological research of K-Ar method were made in the Geological Institute of the Polish Academy of Sciences in Krakow, courtesy of M. Banaś. Research biotite were consulted with Dr. M. Dumańska-Słowik from Faculty of Geology, Geophysics and Environmental Protection AGH in Krakow. Zircon has been separated from charno-ckites and granulites and then analyzed U-Pb age in the Kola Scientific Centre of the Russian Academy of Sciences in Apatity.

3. RESULTS AND DISCUSSION

The **basic granulites** from the Kolvitza region, due to the presence in them large amounts of mafic minerals (Figure 2), have a greenish color. There are massive rock with grano-lepido-nema-toblastic structure, generally random texture, composed of pyroxene (15-20%), garnets (20-30%), plagioclase (0-5%), quartz (0-20%), amphi-boles (15-20%) and ore minerals (up to 5%). Often they are covered of aphibolitization processes. There are also common hornblende, mica, chlorite, epidote. Garnets have a xenomorphic form, are rich in inclusions of rutile, ilmenite, and spinel (hercynite). In the interstycia of these minerals is presence a basic plagioclase, oligoclase and quartz. Plagioclase are generally heavily sericitization. Accessory minerals are rutile, ilmenite, titanite, spinels (magnetite, hercynite), monazite, pyrite and chalcopyrite, and barite, zircon, apatite, iron oxides and hydroxides [8, 9]. These rocks formed by metamorphosed gabbroide intrusions and different sediments [9].

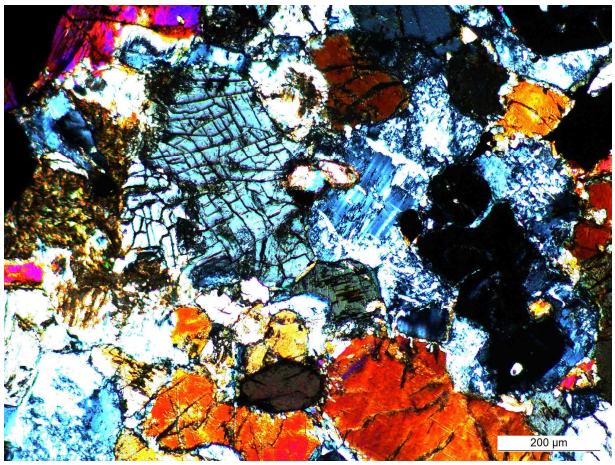

Fig. 2. Microphotograph of the basic granulite (sample 06KG03, crossed pollars).

The age of zircone grains from the Kolvitza granulite (sample 06KG03) determined by the U-Pb metod illustrates the Table 1 (sample weight: 2.0 mg). High compatibility of isochrones illustrated in Figure 3 indicates the age of granulite formation in the range 2362±7 million years. Microanalysis of the studied zircon grains indicate a small addition of Fe and P.

Table 1. Content of the U, Pb isotopes and age of the Kolvitza granulites.

Content [ppm]		Isotopic content of Pb [1)]			Isotopic comparison and age (Ma)			Rho
Pb	U	206Pb / 204Pb	206Pb / 207Pb	206Pb / 208Pb	207 Pb / 235 U	206 Pb / 238 U	207Pb / 206Pb	
86.5	179.5	6000	6.5990	10.323	9.22489	0.441814	2339	0.97
95.3	217.5	7482	6.6334	10.517	8.52010	0.408090	2335	0.96
105.5	273.0	7600	6.6002	12.124	7.59061	0.363571	2344	0.96
117.1	312.0	7544	6.5255	11.337	7.32744	0.350666	2364	0.96

[1)] All comparison correlated by 0.08 mg for Pb and 0.02 mg for U with discriminate mass 0.12±0.04 %.

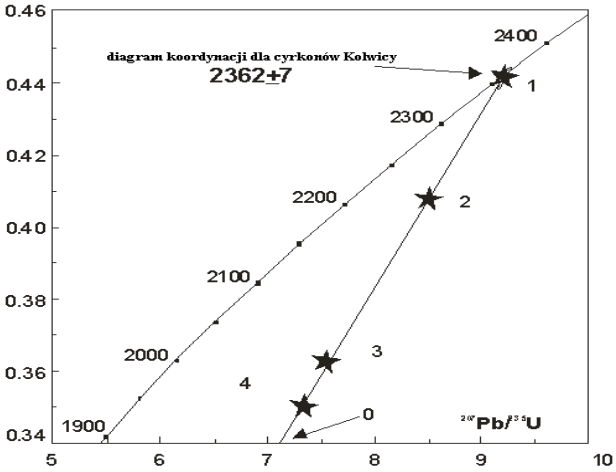

Fig. 3. Isochrones U-Pb for zircons from Kolvitza granulites.

Charnockites are present in the Kolvitza region, cutting enderbites and granulites. Are rich in quartz, feldspars, zircon, garnet and hyperstene. Alkali feldspars are represented by orthoclase and microcline [9, 16]. These gneissitised charnockites-plagioclase-quartz-pyroxene, deep pink color, grano-lepido-nematoblastic structure, direction texture (Figures 4 and 5). They are constructed of quartz (40-60%), alkali feldspar (10%), plagioclase (up to 5%), micas (10%), pyroxene (up to 2%), amphiboles (up to 2%) zirconium and ore minerals (up to 4%). For the most part, they are composed of leucocratic minerals. Charnockites cutting the basic granulites and enderbites. Enderbite from the Kolvitza region are light gray in color, massive holocrysstaline structure, direction texture. They consist of: quartz (40-60%), plagioclase (up to 5%), micas (10%) and pyroxene (up to 2%), amphiboles (up to 2%), zirconium and ore minerals (up to 4%).

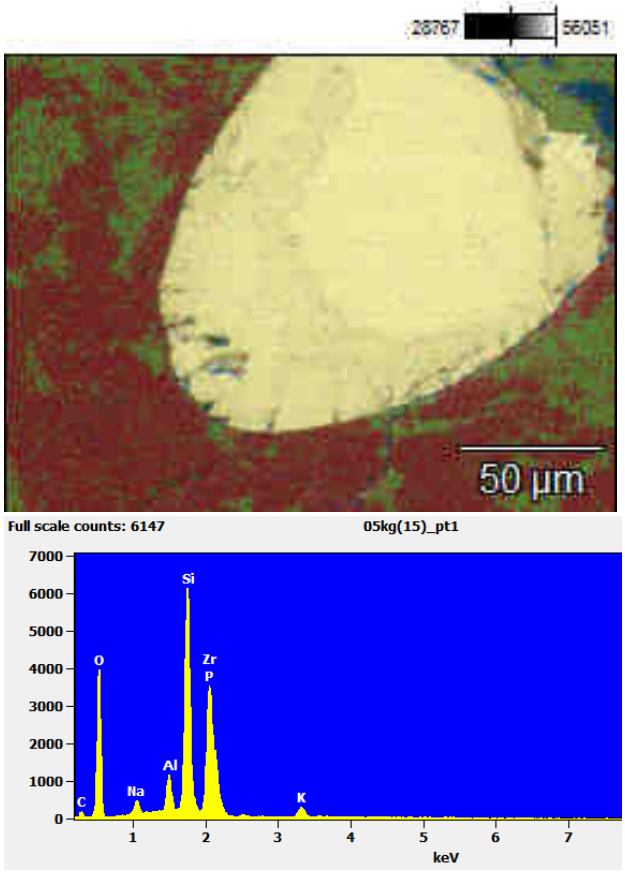

Fig. 4. Phase map of zircon grains near the plagioklases and alkali–feldspar crysstalls using EDS, EDS spectrum of zircon.

In these rocks predominate leucocratic minerals. The accessory minerals are: ilmenite and brookite, and hercynite magnetite, rutile, pyrite, chalcopyrite, hematite, and zircon. Both of these products were probably due ultrametamorphism (melting) of basic granulites. There are present mainly in tectonic thrust belt (mainly in the Kandalakshan), and as rocks associated intrusions in the Kolvitza gabbroide area.

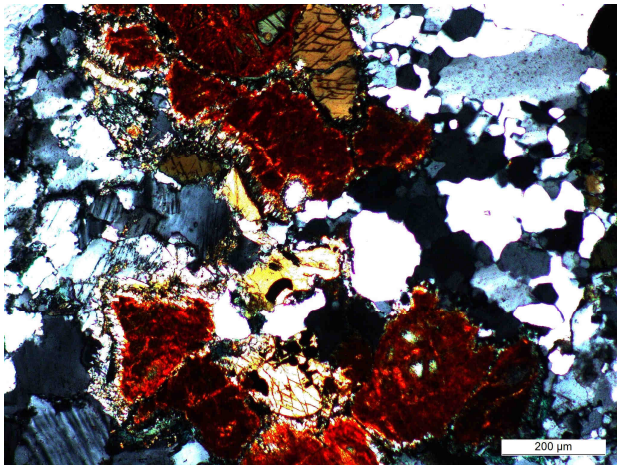

Fig. 5. Microphotograph of the charnockite from the Kolvitza region.

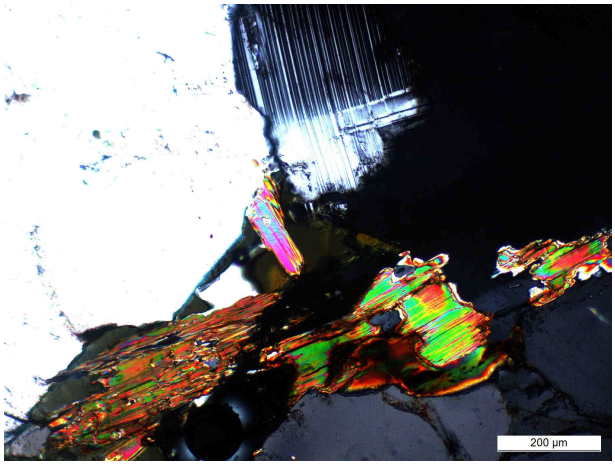

Fig. 6. Microphotograph of typical granitoide veins (ample 18KK02, crossed pollars).

Granitoide veins are two micas - granite, characterized by the color pink or gray, with a thickness generally from a few cm to 0.5 m. They are visible among the garnet amphibolites in the northern slopes of Volosianaya and Zhielieznaya Mounts, as well as in the vicinity of granulite in the area of the top Siennaya Kuriazhnaya, Sriednyj Myss Mounts and in the Kolvitza area. They show the holocrysstaline structure, massive texture, compacted, random or directional gneiss, related to the orientation of mica in the rock. They are constructed of quartz, feldspars (oligoclase, albite) and microcline or orthoclase (Figure 5). Among mafic minerals present biotite and/or muscovite. In these rocks there are also numerous accessory minerals such as sphene, garnet and common hornblende, brookite, pyrite, hematite and goethyte. In these rocks are present the lamina or laminae composed of mica - biotite and/or muscovite. Biotite generally contains high content of titanium, it is often chloritised, sometimes with zirconium grains.

The microscopic observations show that there are two generations micas one edge to the other according to (001). Muscovite (sample 11KK02) is rich in a fengite molecule, or (sample 18aKK03) in ferrimuscovite. The differences in the chemical composition of these minerals are associated with different age and the crystallization temperature (Table 2, Figures 6-8).

Analysed biotites by K-Ar methods indicate the 2823 Ma and 1253 Ma age.

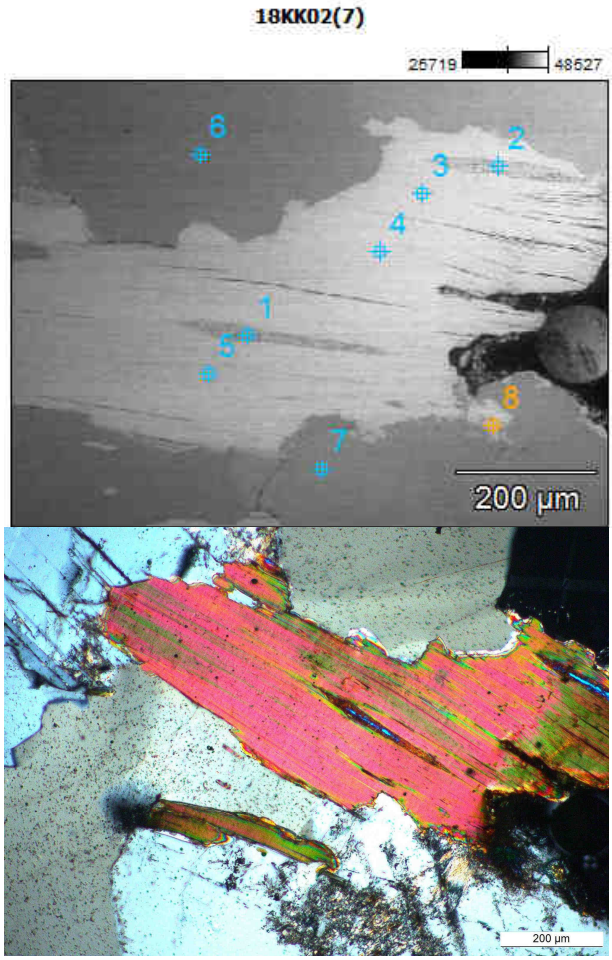

Fig. 6. Microphotographs of biotite-muscovite complex (BSE with point of analysis - right, from polarysed microscope - left).

4. CONCLUSIONS

Age obtained for zircon (also obtained by Kozlov for amphibolites) gives the time of formation and metamorphism LGB Kola in the Kanda-

lakshian region [6, 12, 17-23]. Bridguoter [24] and Barbey and Marth [25] due to the age range of zircons in some of the studies they granulite samples (ranging between 2.7 - 3.1 billion years), point to metasediments of origin of zircons in these rocks. In the case of older granitoide veins components it seems that they are old minor intrusions that are embedded tectonically in other LGB Kola rocks. The veins intersecting amphibolites and granulites indicate the age of magmatic svecofenian processes. These rocks are younger than the Yuvoayviyski massive of the north-western region of the Kola Peninsula and similar granitoids dated to the age of 1950 million years [11], so far considered the youngest rocks associated with LPG Kola. Granitoids are similar to 1644 million years of age, studied by Bernard et al. [26] and the age of granitoids 1431 million years ago, studied by Yudin et al. [27], considered by many researchers so far as products not found in the LPG Kola. K-Ar age of biotite may be underestimated as a result of geochemical processes associated with carbonatite later associations [28].

Table 2. Chemical composition [wt %] of elements in the micas from granitoide veins, measured by EDS.

Sample	C	O	Na	Mg	Al	Si	K	Ca	Ti	Fe
18KK02(7)_pt1 muskovite	4.50	1.73	0.65	2.00	0.46	2.18	1.56	1.02	0.40	5.13
18KK02(7)_pt2 muskovite	4.43	4.85	0.99	1.31	0.47	1.65	0.92	1.84	0.21	3.34
18KK02(7)_pt3 biotite	3.70	1.93	0.88	5.95	8.71	0.92	6.18		0.84	0.62
18KK02(7)_pt4 biotite	3.72	3.17	0.84	6.10	8.29	0.19	6.09		1.00	0.59
18KK02(7)_pt5 biotite	2.99	2.88	0.79	6.29	8.24	0.72	6.19		1.09	0.80
18KK02(9)_pt6 muskovite	2.56	3.22	1.52	5.30	6.10	3.77	0.55	7.22	0.31	9.46
18KK02(9)_pt7 muskovite	2.61	3.46	1.38	5.28	5.67	3.89	0.58	7.10	0.26	9.77
18KK02(9)_pt8 muskovite	2.76	4.53	0.80	1.87	8.43	1.67	0.19	1.29	0.28	8.19
18KK02(9)_pt9 muskovite	2.82	3.24	1.38	4.50	5.87	4.25	0.66	6.91	0.56	9.82
11KK02(2)_pt5 biotite	2.84	5.98	1.25	0.78	2.25	6.09	7.10		0.53	3.19
11KK02(2)_pt8 biotite	2.72	5.10	1.23		9.09	1.38	0.50			

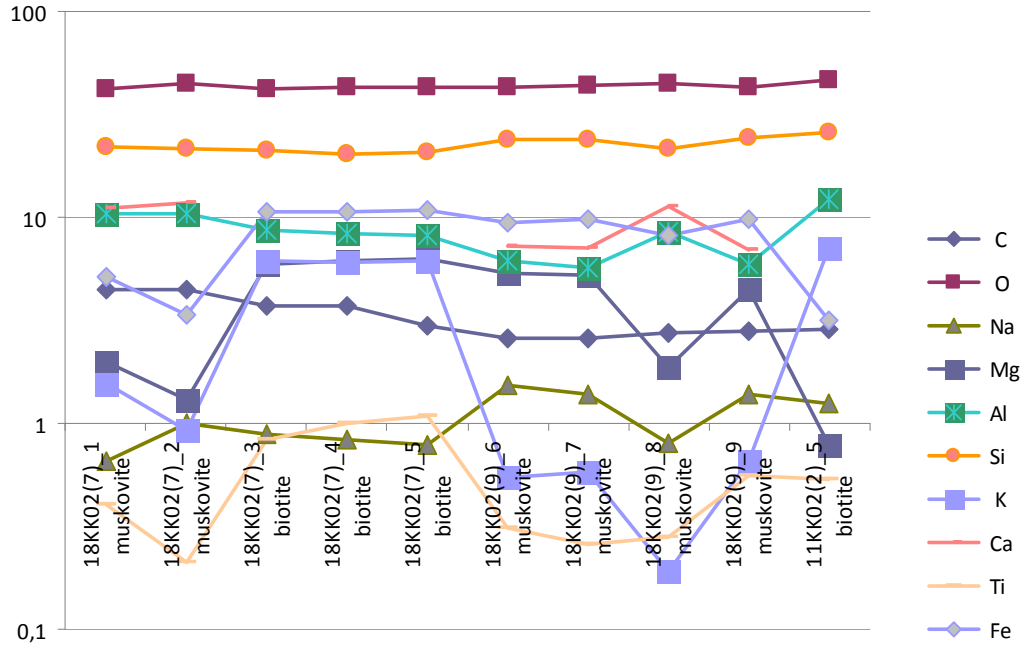

Fig. 7. Distribution of elements in studied micas.

AUTHORS' CONTRIBUTION

Conception and design: MAH; Development of methodology: MAH, FPM, TBB; Acquisition of data: MAH, TBB, NAE, PAS; Analysis and interpretation of data: MAH, FPM; Writing, review and/or revision of the manuscript; Administrative, technical, or material support: MAH, TBB, NAE, PAS; Study supervision: MAH, TBB. All authors are involved in drafting the manuscript, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Mathematics planimetry map model of diversity and petrology in the Kandalaksha part of Lapland Granulite Belt (Kola Peninsula, NW Russia)

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ABSTRACT

In the Kola Peninsula, near the Kandalaksha region are exposed the Lapland Granulite Belt (LGB) rocks with present of the numerous of metamorphic rocks types, which are a paleo-colission zone between Kola and Belamorian blocks. They have a ortho- and paraprotolith origin. Orthometamorphic rocks are mainly metavolcanites (garnet amphibolites) and metaintrusives (metamorphosed in the amphibolite and granulite facies). Parametamorphic rocks are different sediments. These complexes are cut by different of veins rocks as a different secondary processes corresponding with major tectonics stages firming of the northern Baltic shield. These rocks were examined using petrographic methods of mathematical analysis and mathematic. Results allowed showing the intensity and extent of the various processes occurring in the rocks of LGB. In conclusion it should be emphasized that these rocks have many changes both secondary and metamorphic that overlapping rubbing contributed parageneses older.

Key words: Mathematics geology; Planimetry; Fractal analysis; Lapland Granulite Belt.

INTRODUCTION

The subject of this study are the paleo-proterozoic rocks of Kandalakshan part of the Lapland Granulite belt of Kola (LGB), which are visible on the White Sea in northern Scandinavia (Fig. 1). There are is a metamorphosed collision zone between Kola and Belamoryian blok age of 2,4-2,8 Ga [1-11].

In the kandalakshan part of LGB are present the following types of metamorphites: amphibolites, crystalline schists, garnetites, metamyllonites, granulites, charnockites. These are a product of ortho- and parametamorphism.

Orthometamorphic rocks are mainly metavolcanites (garnet amphibolite) and metaintrusives (metamorphosed in the amphibolite and granulite facies [12]). Parametamorphic rocks are different sediments [13]. They are cut by different of rocks veins. In these rocks were found a relict textures and minerals. As the process of metamorphism occurred for corrosion and replace the minerals of the older generation by the younger (eg. quartz in prehnite veins [14]). Visible are paragenesis characteristic facies and granulite facies metamorphism lower level. Also indicate these rocks different secondary processes such as hydrothermal, especially in the vicinity of the veins.

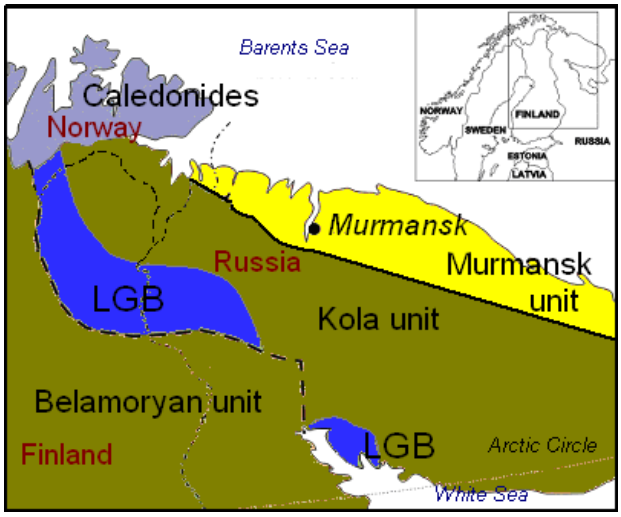


Fig. 1. Localization of Lapland Granulite Belt on the Balic Shield (based on Lauri et al. [15], Pozhilenko et al. [16] and author's studies).

2. MATERIALS AND METHODS

Rock samples collected by author have been petrological analysed using an optical and an electron microscope with EDS attachment. In developing the research results were used also previously published results of geochemical, isotopic, geochronological analyzes [17]. These studies were carried out in the Laboratory of Electron and Optical Microscopy Department of Geology and Protection of Lithosphere at the Department of Earth Sciences and Spatial Management UMCS with an optical microscope Leica DM 2500P and Scanning Electron Microscope Hitachi SU6600. These results are developed mathematically using software SURFER, located in the Academy of Mining and Metallurgy in Krakow and mathematical tools associated with Microsoft Office. Analyses were performed using the box-counting fractal dimension analysis [18, 19].

3. RESULTS

Distribution ratio of color in the study area shows map 1. On its basis, that in the eastern part of the investigated area are both rock brighter and darker. The rocks, which prevails part of melanocratic minerals (mostly garnet amphibolites), located mainly in the upper parts of Kandalaksha Tundra region. However, in the valleys dominated by rocks predominantly bright color. There are various types of schists, in which the large proportion of quartz and feldspars are. Analysis of these precipitates shows that in this area of amphibolite facies rocks (metavolcanites and metasediment) are in the form of alternating layers of maturing at a slight angle toward the north. These slopes of Kandalaksha Tundra Mountains is constructed of metavolcanic material with addition of matasediments. These rocks formed from volcanic material are more resistant to weathering, form blocks and walls in the slopes of the mountains, and less resistant metasediments form valleys [13]. Tectonic and glacial activity in the region has contributed to sharpen the morphological forms of negative (Fig 2).

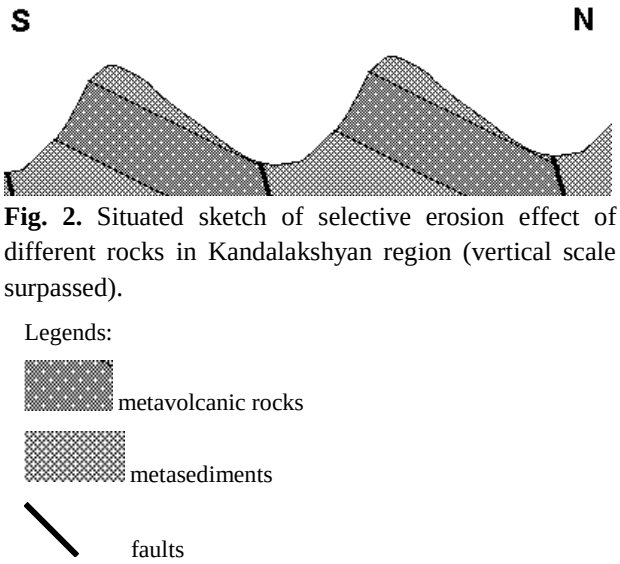


Fig. 2. Situated sketch of selective erosion effect of different rocks in Kandalakshyan region (vertical scale surpassed).

In the north-eastern part of the study area granulites occur in zones close proximity of mineral veins (eg. in the north-eastern slope of the Sriednyj Myss mount). Analysis of the spatial distribution and characteristics of individual minerals indicate their different populations, which is closely associated with varying degrees of intensity and range of metamorphic processes. Examples are pyroxene occurring in the rocks of LGB Kola (Maps 2-6) illustrates the distribution of field participation % vol. clinopyroxene, and map 2 - orthopyroxene. In analyzing of these maps (2, 3) can be seen that the occurrence of amphibolites are in the western part of the study area, pyroxene they hardly occur (especially orthopyroxene). The exception is the area of overlap, found in the northern slopes of Zhieleznaya mountains where there pyroxene-

garnet eclogites, with the highest concentration clinopyroxene whose share exceeds 30% of the rock volume [8]. There are much more than granulites, situated in the north-eastern part of the study area, where the predominant orthopyroxene (about 25% vol.). Most rich clinopyroxenes are granulites wchich are metamorphosed sedimentary rocks (quartz-rich metasandstones with a conspicuous premetamorphic lamination [17]. Metagranulites derived from igneous rocks (metagabbros) are richer in clinopyroxenes [15, 20-23].

Dependence illustrated in Figure 3 shows several varieties of amphibolites rich in pyroxene. The first of these is characterized by a predominance of othopyroxenes over clinopyroxenes and a substantial share of the amphibole. Represent the variation of the amphibolites and granulites. The second variant is one of the rocks, which have a larger share of clinopyroxenes over othopyroxenes, sometimes rises in them amphibole. The advantage, however, rocks poor in amphibole. These granulites, which only a few core areas have small amounts of secondary amphibole. Trend set in bold lines indicates that these rocks dominate othopyroxenes under the clinopyroxenes.

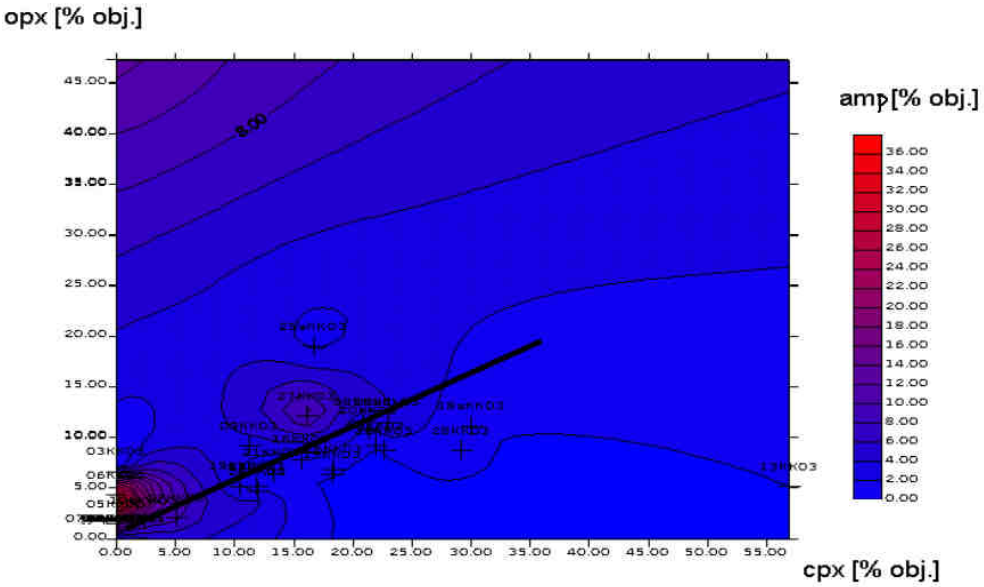


Fig. 3. Comparison of content of pyroxenes and amphiboles in the LGB Kola.

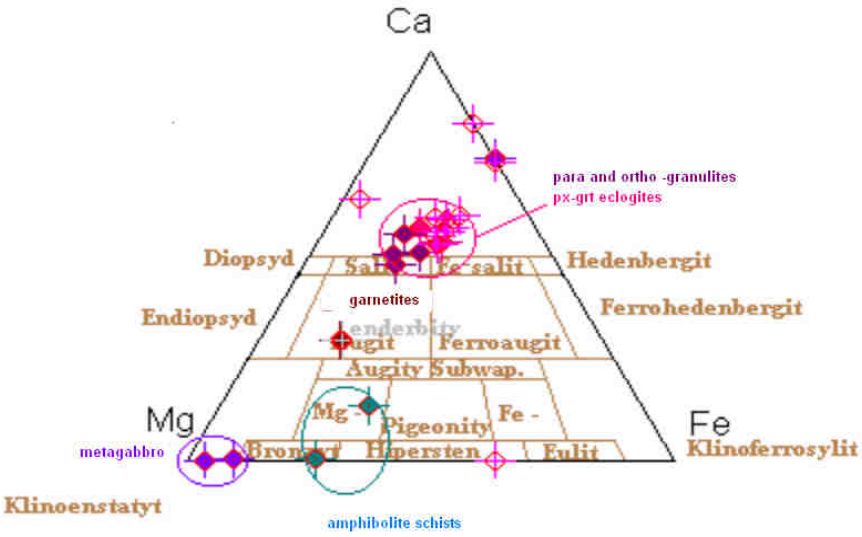


Fig. 4. Triangle classification of pyroxene (by Bolewski and Manecki [24]) based on the EDS analysis.

Pyroxene classification based on the EDS micranalysis is shown in Figure 4. Analysis of this diagram indicates a high proportion of salites and ferrosalites, especially in granulites and eclogites. In the basic granulites dominated by salites calcium-magnesium, in white granulites: calcium-ferrous. Moreover, in the white granulites is present a hipersten acidic and rich in calcium pyroxene. In metagabbro is visible mainly clinoenstatite, in the amphibolite schists: magnesium pigeonite and bronzite. It is interesting location field augite, which is present in enderbites and garnetites. Location these minerals in the triangle classification allows to establish a relationship garnetites with harzburgite-charnockite line [25]. Given the significant nonsaturated of silica of garnetites should be noted that they are the most basic element of this series. Augite it was founded in granulites as a relict mineral surrounded by a ringed reactionary formed in secondary processes. In light of these data it can be hypothesized that garnetites and minor intrusions of enderbites that occur among amphibolites, were metamorphosed under similar conditions. Maps 4-6 show the distribution of ions of calcium, iron and magnesium in piroxenes. The highest accumulation of calcium in these minerals are in the rocks of the Siennaya Kurtiazhnaya mountain and in the north-eastern part of the studied area. It overlaps with part of the granulite occurring there. Relatively much calcium is present in the rocks of the overthrust zone in the western part region. In the area of this zone, as in the eastern part of the Sriednyj Myss mountains, comes to the discharge of calcium from the granulite. This occurs by the action of the hydrothermal metasomatosis. Analysis of the map 4 shows that the Kandalaksha area is poorer in calcium and richer in iron (Fe^{2+}). There are metavolcanites exposure. From the triangle pyroxene classification, (in Figure 4) shows that in the amphibolite metagabbro and amphibole schists, the pyroxene are most enriched in Mg. Distribution of amphibole in rocks of the studied area is shown on Map 7. The largest number of these minerals are concentrated in the rocks of the western part of these region. The biggest share of amphibole have a amphibolites arising from the volcanic rocks that make up the upper parts of Kandalaksha tundra. In the valleys, where the predominant metasedimentas schists, amphibole participation in them is much smaller. In the north-western part of the study area amphibole content in these rocks is smaller, especially in granulites, in contact with the mineral veins. Increasing the share of amphibole in the area northeast of the mountains Sriednyj Myss is associated with amfibolizing granulite. Lying at the Siennaya Kurtiazhnaya (northern slopes) metamyllonites and plagioclase schists characterized, in relation to granulite, increased amounts of amphibole. Well-developed myllonitization (tulip structure - fault tectonics) causes the amount of amphibole in it caused is much smaller than in the rocks of the kandalakshyan region, because they are often there chloritization. Interesting is the relative juxtaposition of amphiboles share garnets and quartz in these rocks (Figure 5).

Analyzing the diagram on the Fig. 5 it is present a several genetic types of studied rocks. Visible are rocks rich in garnets and quartz -poor in amphibole. These granulites and eclogites. It also includes metagabbros. The second type are rocks richer in amphibole and quartz. There are various types of crystalline schists (metasediments), building of the valley in the region of Kandalaksha and amphibolitizing granulityes, in the transition zone (towards lower facia) in the north-eastern slopes of Sriednyj Myss Mount, as well as associations mineral feldspar-amphibole schists. The next type are rocks rich in amphibole and poor garnets and quartz. These are massive amphibolites (metapelites, pyroclastic origin). Outcrops of these rocks are located in the slopes of the Kandalakshska Tundra mountains, in direct contact of metavolcanites [20, 23, 26-28]. Rocks that are rich in garnets and amphibole, with varying degrees of quartz are metaamphibolites, which are also located in areas of Kandalaksha Tunda region, which consists of two types: rich with garnets and amphibole and lower contents of quartz, and a slightly higher content of quartz and amphibole at the expense of garnets.

Quartz in these rocks may be a secondary product, metamorphic (this is confirmed by cathodoluminescence observations), resulting in the dissolution of feldspars in high-grade metamorphism. Finally metagabbroides are rich in amphibole, with minor contributions of garnets and quartz, which followed the classification diagram in

the lower right corner. In these rocks represent a much larger share of feldspars (Figure 6). Analyzing Figure 6, it is noted that there are in the area of research rocks rich in garnets, but poor in amphibole and feldspar (mainly granulites) and rocks rich in amphibole and garnet, quartz-poor (mainly amphibolite-metavolcanites). Relatively rich in feldspar, and amphibole and garnest all kinds of crystalline schists, while the highest content of feldspar, with little participation of garnets and amphibole, are metagabbros [15, 21-23].

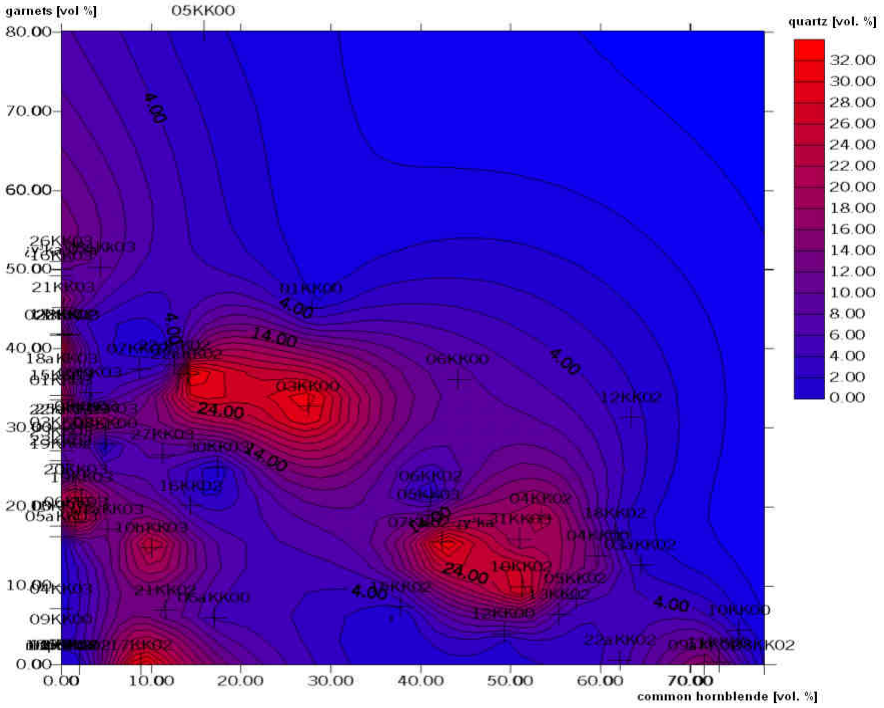


Fig. 5. Comparison of the content of amphiboles between quartz and garnets in studied rocks (vol. %).

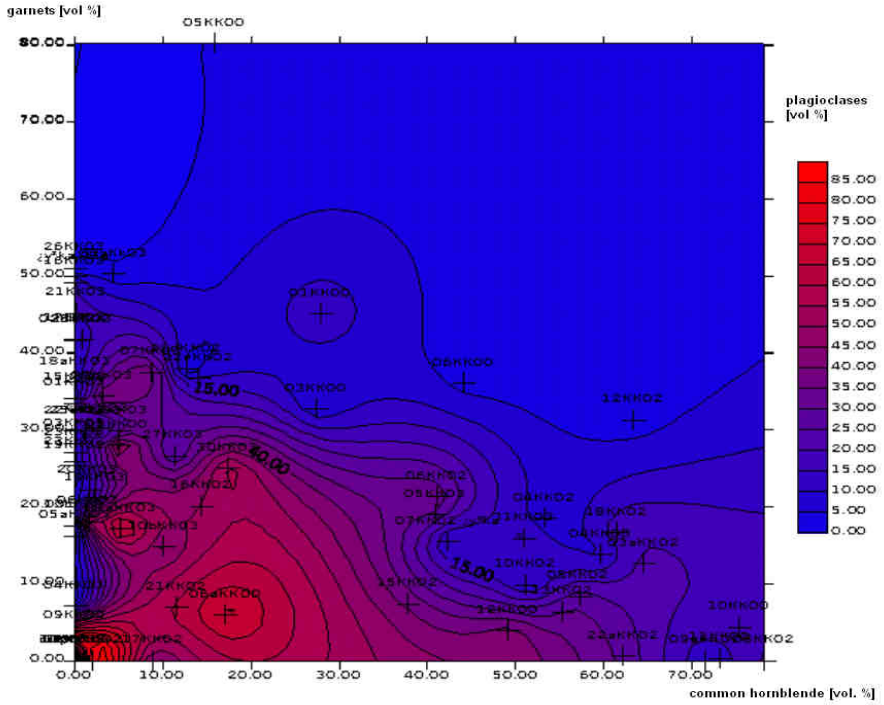


Fig. 6. Comparison of the content of amphiboles between plagioclases and garnets in studied rocks (vol. %).

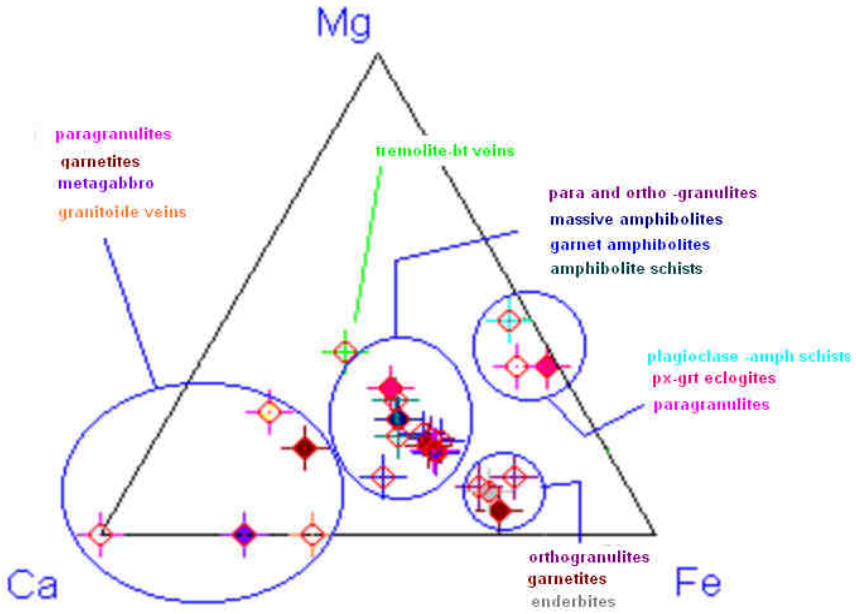


Fig. 7. Triangle of amphibole classification developed on EDS analysis.

Classification of amphibole in the studied rocks illustrated in Figure 7 [24, 25]. Analyzing the amphibole classification triangle (Figure 7), it is noted that most of the iron, calcium and magnesium containing amphibole from amphibolites of Kandalaksha region (western part of the study area) those that arise in the process of secondary in granulites. This suggests that amphibole from amphibolites (metavulcanites) from Kandalaksha tregion were formed at the expense of pyroxene, as much later then granulites. It is also important to note that the position of the amphibole granulite basic classification of the triangle coincides with the position of amphiboles and garnetites enderbites, tschermakite rich molecule. Overlapping of these fields for garnetites and enderbites confirms the hypothesis of a common metamorphism, as set out above, the analysis of pyroxene (Fig. 5). It's the same position amphibole points of the triangle (Figure 7) of the basic granulite and enderbites indicates that these amphibolitization of rocks occurred with the participation of permeating components of cutting them mineral veins. The two types found in eclogites that occur feldspar shale. They were created at the expense of pyroxene. Amphibole low in magnesium second type formed in the same ambient eclogite rocks. Type the amphibole-rich in tremolite molecule, occurring frequently in granulites and metagabbro, is probably associated with the crystallization process granitoide veins and tremolite-biotite (found in them like amphibole, Figure 7).

Maps 8-10 show the distributions of calcium, magnesium and iron in amphiboles. The highest calcium content (map 8) exhibit granulite and amphibolite rocks of Kandalaksha region of northern slopes of the Zhielieznaya mountains. Increased content of the tremolite particles in amfiboles in these rocks is associated with the dissolution of calcium pyroxene in granulites and the effect of the impact of veins rich in tremolite. Amphibole rich in calcium are also members of the locally occurring rock (in the vicinity of the veins), which are the product of retrogressive metamorphism (epidote-amphibolite facies). The least calcium present in metamyllonites and amphibolites from the Kandalaksha region. They are the product of metamorphism volcanics and sediments under amphibolite facies (maps 9 and 10). Most iron in amfiboles located in the upper parts of the Kandalaksha mountains, especially around peak Wolosianaya and the northern slope of the mountain Zhielieznaya and in the amphibolites feldspar schists, metamyllonites and the northeastern part of the study area, the northern slopes of the Sriednyj Myss and Siennaya Kurtiazhnaya mountains. The comparison of 9 and 10 maps, metavolcanites differentiation in terms of the

distribution of iron and magnesium in the upper parts of the Kandalaksha mountains. The relatively high content of magnesium and iron found in aftergranulite amphibolites from the northern slopes of the Sriednyj Myss peak. This diversity in Kandalaksha region metabvolcanites allows us to distinguish among them two types occurring in this area. Metavolcanites predominate, in which are rich in amphibole tschermakite molecule with are richer in magnesium.

The occurrence of biotite (map 11) in the studied rocks indicates several populations of this mineral. In the diagram shown in Figure 8 shows that there are two types of biotite. The first is found in amphibolites, which contains a high content of titanium. The second type is biotite core origin. It is found in small intrusions metamorphosed granitoide age of 2.4 billion years [1, 3, 4-7, 9-11, 19, 29, 30]. There is also much younger biotite, poor in titanium, the origin of the secondary and the core, mainly of the younger granitoids.

Distribution of participation (% vol.) garnets in these rocks shows a map of 12. Relatively most of these minerals present in the rocks from the Kandalaksha region, where are exposure the metavolcanites and granulites of the north-eastern part of the Sriednyj Myss mountain, arising from gabbroic metamorphic rocks. Significantly fewer of these garnets occurs in amphibolites (metasediments) of the Kandalaksha valleys and northern slopes of the Siennaya Kurtiazhnaya and Sriednyj Myss mountains, where the shale feldspar and metamyllonites. Location of the results of analyzes of these rocks garnets triangle classification shows Fig. 9. He points to the existence of several types of garnets.

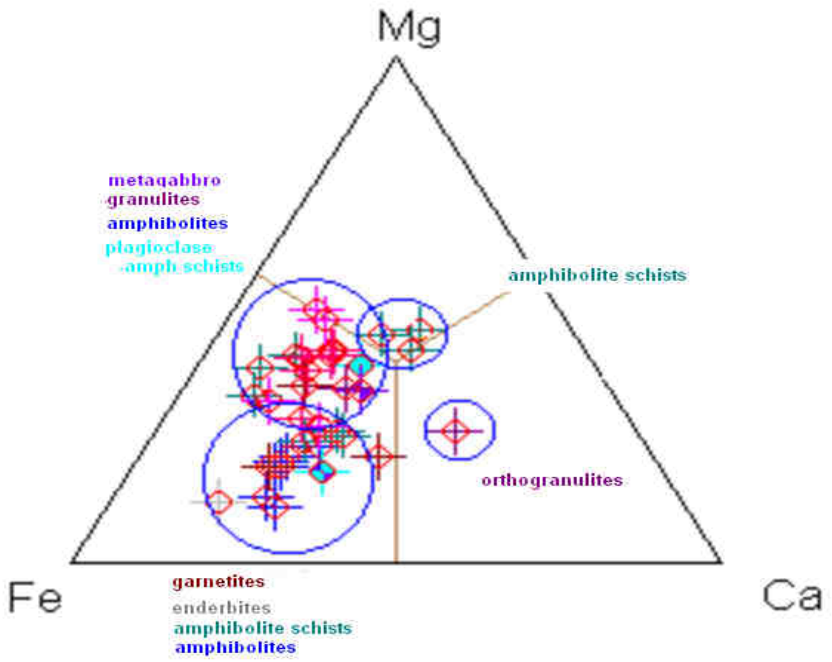


Fig. 9. Triangle garnets classification based on the EDS analysis (vol. %).

Most magnesium rich garnets its present in white granulites, metagabbro and in some amphibolite schists. The poorest in this component are garnets from amphibolites and feldspar schists. In metavolcanic rocks represented by amphiboles exist garnets containing the larger part of iron ions. Mössbauer analyzes indicate that iron is usually present in dodekaedric position (approximately 90%), corresponding to the part of Fe^{2+} (almandine molecule) and only 5% of iron Fe^{3+} in the octahedral positions (andradite molecule [12, 31]). Partially distribution of magnesium and iron are also held within the micas. Some garnets have in their structure increased the number of Ti^{4+} ions. Another garnet population is enriched in calcium. It occurs in granulites that undergoes secondary as a result of an interaction of hydrothermal veins. The projection classification (Figure 9), as in the

case of amphibole and pyroxene, the distribution of characteristic points for garnets from garnetites and enderbites coincide, indicating similar conditions of metamorphism.

Distribution of ions in these minerals and their distribution in the area illustrated in the maps 13-15. Analysis of the distribution of calcium ion concentration indicates its largest in garnets from acidic granulite from the north-eastern part of the study area (top Sriednyj Myss and Siennaya Kurtiazhnaya). A large amount of calcium present in para-granulites and amfibolizing granulites in the transition zone to amphibolite facies (diaphtoretic). In the Kandalaksha area high concentration of calcium takes place in a part of the mountain peak Volosianaya (metavolcanites) and in the zone of overlap among the eclogites. Poorer in calcium are mainly of garnet amphibolites from south-western slope of the mountain Volosianaya, and from the rocks to the east of the ridge top of Zhielieznaya (metasediments). Analysis of the share of iron garnets (map 14) shows increased participation of this element in these minerals thrust zone (increased metamorphism) and in some granulites. Definitely poorer in iron garnets from amphibolites are both from the Kandalaksha area and from the northern slopes of Siennaya Kurtiazhnaya and Sriednyj Myss mountains. Map 15 shows the distribution of the share of magnesium garnets. Most of this element is concentrated in the metavolcanic rocks of top of Zhielieznaya, and in the granulite of southern part of the Sriednyj Myss mountain and amphibolite sschists of the northern slopes of the Siennaya Kurtiazhnaya and Sriednyj Myss mountains. The relatively poorer in magnesium are para-granulites surging west from the top Siennaya Kurtiazhnaya, and east from the top Sriednyj Myss. Similarly, poorer in magnesium are the metasediments rocks of the northern slopes of the Zhielieznaya and Volosianaya Mts.

Distribution of feldspars in the studied rock samples shows the map 16. Most of these minerals is within some granulite (intrusions intersected by granitoides) in the vicinity of the top Siennaya Kurtiazhnaya and near the impact zone of albite-prehnite veins at the Sriednyj Myss. In the Kandalaksha region the content of plagioclase is higher in the overlap zone, and in the meta-myllonites and metagabbro, in the para-amphibolites and in the same surrounding rocks with Volosianaya advance. Least feldspar occurs in metavolcanites in the peak of Kandalaksha region, as well as granulites unchanged. Figure 10 shows the classification of a triangle in the samples tested feldspar rocks.

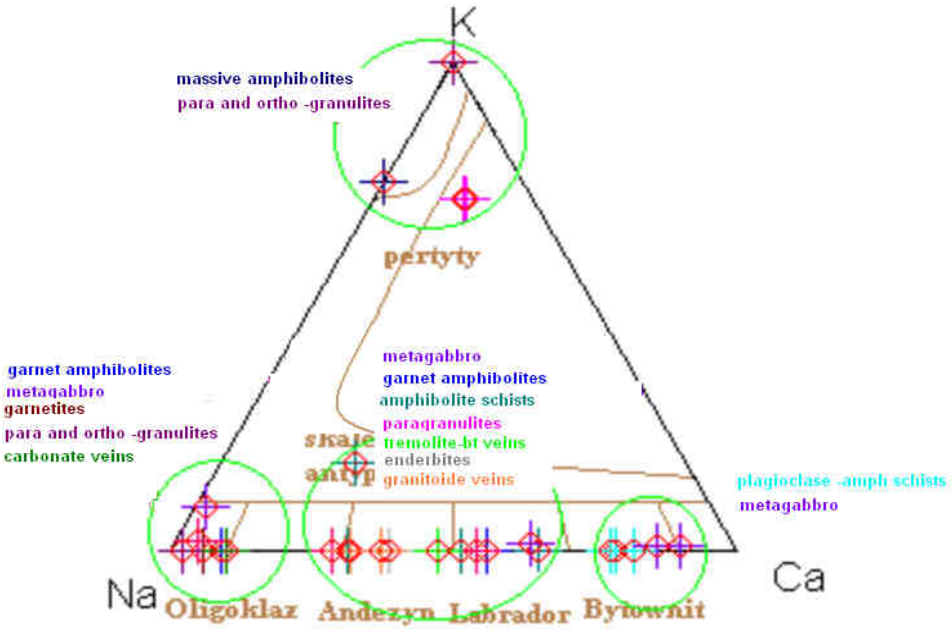


Fig. 10. Triangle feldspar classification based on the EDS analysis (vol. %).

In a triangle that can distinguish several varieties of plagioclase. One of them is a basic plagioclase-rich in An molecule which are present in metagabbro of Kandalaksha region and feldspar-amphibolite schists of northern slopes of the Sriednyj Myss and Siennaya Kurtiazhnaya mountains. The very nature of feldspars in both of these rocks suggests that like their origins, which has been largely obliterated by metamorphism in amphibolite facies (mainly in metagabbro) in the vicinity of the impact zone of hydrothermal solutions.

Another generation of plagioclase are secondary type, occurring mainly in the amphibolite and granulite rocks and adjacent enderbite, granitoide, and tremolite-biotite veins. These are characteristic of plagioclase amphibolite facies. Albite occurs in both amphibolites and in granulites. The origin of this mineral is associated with the formation of prehnite-albite veins. Conditions for the crystallization are characteristic prehnite-chlorite veins. Under similar conditions, the physico-chemical crystallized potassium feldspar present in granulites (para and ortho) and amphibolites in the vicinity of granitoid veins. Maps 17 and 18 provide more detailed information on the association between feldspars with the nature of the rocks studied. The share of basic plagioclase is the most exposed among the eclogites and amphibolites in the northern slope of the northern Zhielieznaya mountains, and granulites and feldspar schists, occurring in Siennaya Kurtiazhnaya and Sriednyj Myss massifs. Much poorer in alkaline rocks are plagioclase from amphibole of Kandalaksha region and amphibolizing granulites from the area of the top Sriednyj Myss. The share of acidic plagioclase (map 18) is more variable and is closely associated with neighborhood feldspar veins (up Volosianaya), granitoides (Top Zhielieznaya), albite-prehnite veins (Kandalaksha region and top Sriednyj Myss and Siennaya Kurtiazhnaya) and tremolitowo-biotite veins (Sriednyj Myss). This map also indicates the secondary nature of acidic plagioclase, whose share rises significantly in rocks from the interface veins rich in acidic plagioclase and alkali feldspars. Distribution of quartz in rocks within the studied area is illustrated on the map 19. It indicates clearly increase the share of quartz in para-amphibolites of Kandalaksha region, builders valley, and especially in the Volosianaya mountains and in some para-granulites in the area Sriednyj Myss and Siennaya Kurtiazhnaya.

It is interesting to demonstrate that select trends in these rocks, visualized in diagrams (Figures 11-14). They represent the pyroxene part relative to feldspar and quartz (Figures 11-12) and the share hornblende and biotite with respect to quartz and feldspar (Figures 13-14).

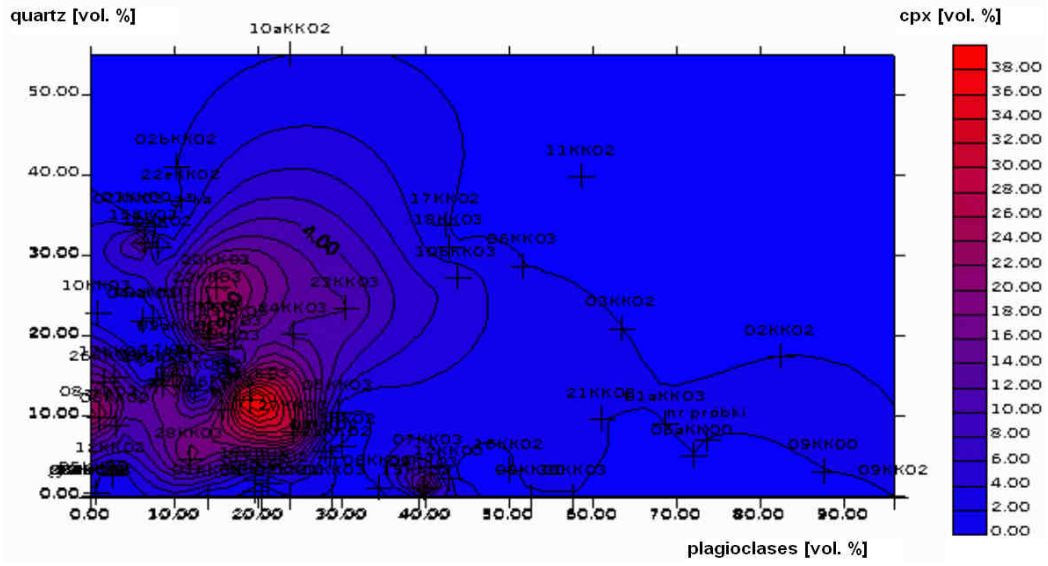


Fig. 11. Comprising of the content of clinopyroxene with plagioclase and quartz (vol %).

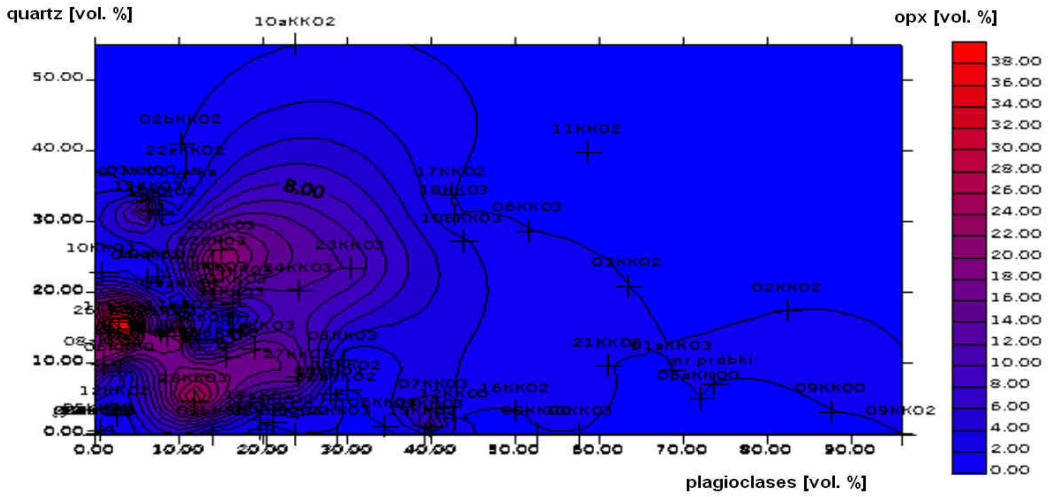


Fig. 12. Comprising of the content of orthopyroxene with plagioclase and quartz (vol %).

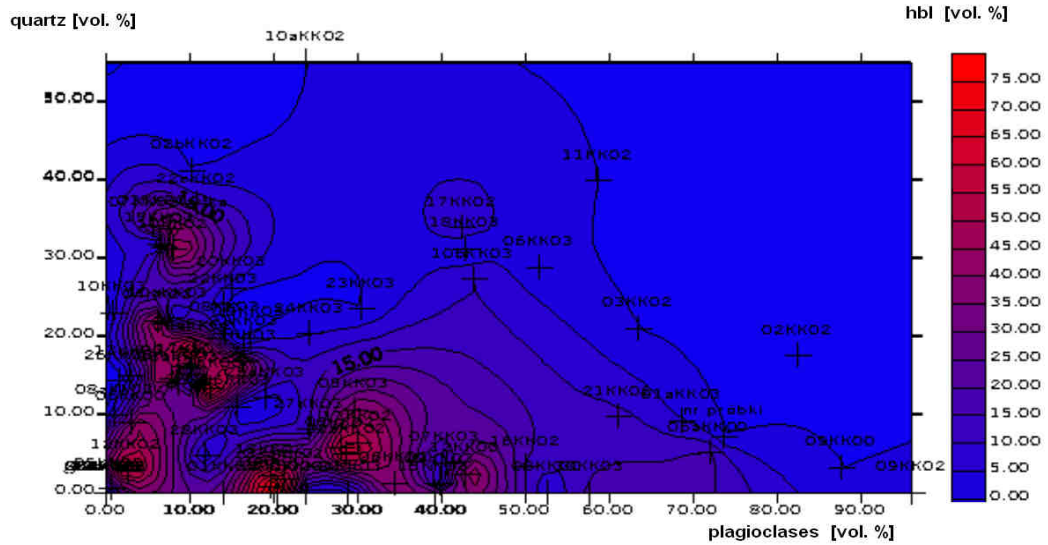


Fig. 13. Comprising of the content of common hornblende with plagioclase and quartz (vol %).

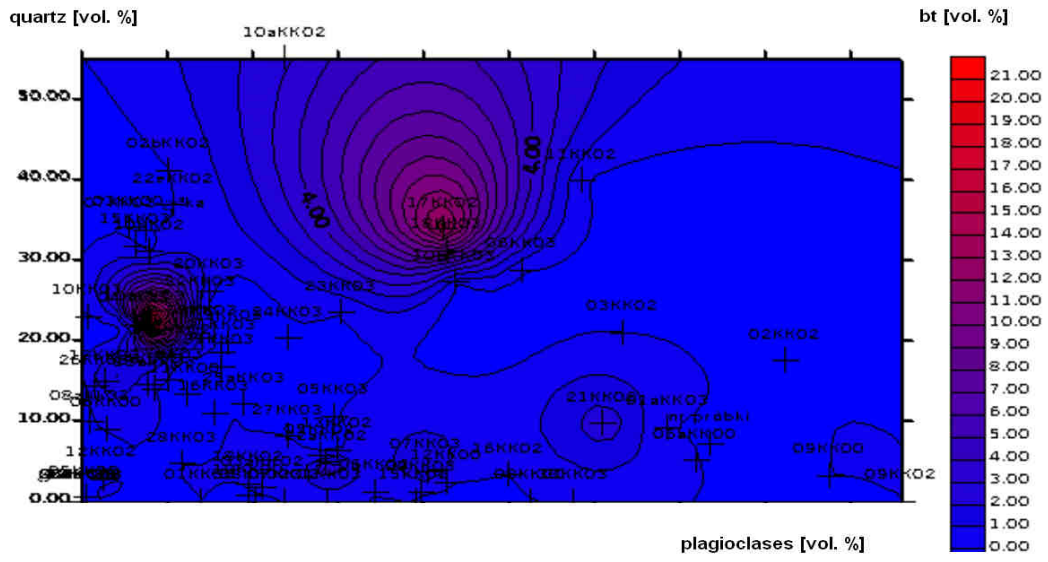


Fig. 14. Comprising of the content of biotite with plagioclase and quartz (vol %).

Figure 11 notes analyzing the two types of rocks. Rocks that are rich in feldspar (mainly secondary changed and para-amphibolites and metagabbros, the second type is a rock rich in quartz metamorphic origin. Rocks that are rich in clinopyroxene divided into two groups: richer in quartz – in eclogites and garnetites where quartz was probably at the expense of feldspars as a result of high-grade metamorphism and rich in quartz and clinopyroxenes-granulites of sedimentary origin. The second group consists of rocks rich in feldspar. There are various kinds of metagabbro. Figure 12 shows part of quartz and feldspar compared to the content orthopyroxene. Analyzing this diagram states that rocks rich in quartz are sedimentary origin. Rocks from the zone with elevated orthopyroxene (granulites richer in feldspars, Figure 13) are rocks in which the pla-gioclase are related to the impact of the processes associated with the formation of veins (maps 14, 18). Analysis diagram 14 shows a clear separation of the amphibolite rocks (mainly metasediments) rich in quartz. The second distinct component, which in this diagram, it is noted that amphibolites (secondarily altered plagioclase and shale mountains Sriednyj Myss and Siennaya Kurtiazhnaya) which are rich in feldspar. Increased content of amphibole among some granulite metamorphism is the result of retrogresive. This takes place in the vicinity of the mineral veins. Diagram 13 shows that most of biotite occurs in acidic rocks rich in quartz (granitoide veins) and among the amphibolite rocks (paraschists). A slight amount of this mineral occurs in rich in feldspar metagabbro (rocks rich in plagioclase). Other minerals such as chlorite in more concentrated in the top region of northern slopes and Siennaya Sriednyj Kurtiazhnaya Myss (map 20), and where there are in metamyllonites in the Kandalaksha region. The rocks of the northern part of the mountain top Zhielieznaya consist mainly of metagabbro rich in titanium. In the Kandalaksha area as well as in the north-eastern part of the study area was found secondarily altered rocks enriched in prehnite (in the vicinity of the veins).

Characteristics of chlorites from studied rock samples is shown in Figure 15. In metagabbro, para-granulites and amphibolites (metavolcanites), usually secondarily altered chlorite are rich in iron and aluminum. They are also in a tremolite-biotite rock. Are genetically associated with hydrothermal activity prehnite-albite-carbonate zone [17]. In metagabbro is present clinochlor. Eclogites as well as some para-amphibolites contain chlorite rich in Fe.

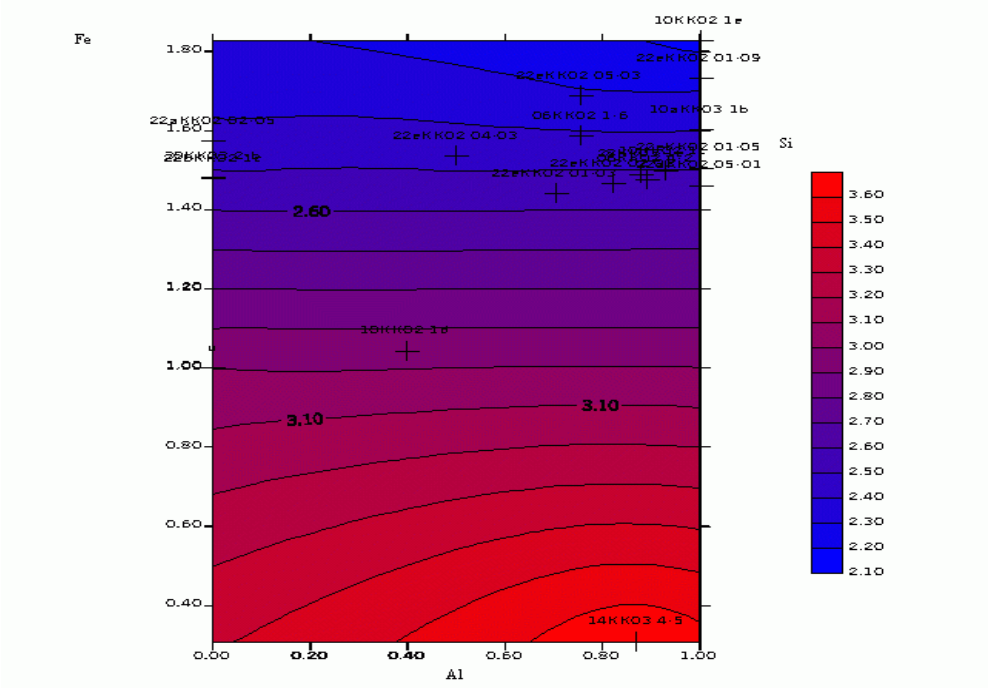


Fig. 15. Chlorite in LGB rocks identified basis of EDS analyzes.

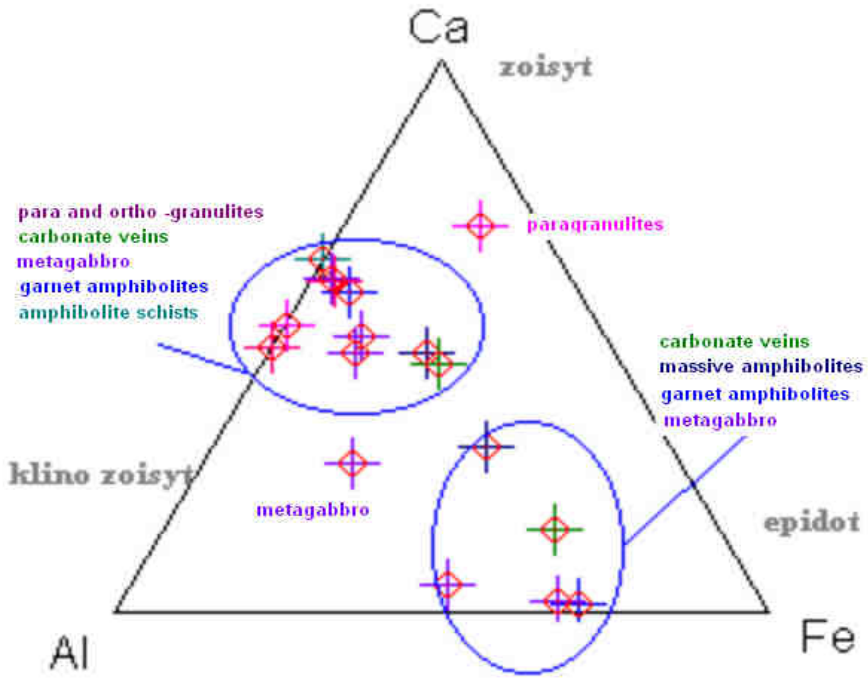


Fig. 16. Triangle classification of minerals from the epidote group based on the analysis point by EDS.

Epidote group minerals in the studied rock samples are grouped into two types (Fig. 16). They are clinozoisite-zoisite present in the amphibolite and granulite rocks, epidote, found in prehnite-albite veins and some amphibolites and metagabbro. In the gabbro also often occurs clinozoiste.

Titanium minerals are common among the rocks in the Kandalakshyan area of LGB Kola, numbered among the Petrov [32] for the province of ilmenite-titanite. They are usually grouped in the titanite, occurring mainly in the amphibolite rocks and in some granulites affected by the diaphtoreza. Concentration of rutile occur mostly in the acidic granulite and rocks richer in garnet, which occurs as infix. Ilmenite is characteristic mainly for basic granulite and metagabbros and garnetites. In some samples (granulites acidic and basic) were also found occurrence chromite, chromium hercynite and garnets (knorringite - identified by XRD).

Analysis of ore minerals in these rocks (Table 1) demonstrate the presence of certain trends. Minerals most frequently coexists with the titanium apatite, mainly in granulites and amphibolites. In close proximity of the prehnite-albite veins are found rocks enriched in REE inclusions. In all types of rocks are iron oxides and sulphides, also found in the veins of ore and granitoid (especially those dating at 1.1 Ga [31]). Fractal analysis were subjected minerals pyroxene, amphibole, garnet and selected quartz crystals. Pyroxene analysis showed that the lowest rate of fractal dimension exist among some granules and, pyroxene-garnet eclogites [17]. The highest fractal dimension are pyroxene transition zone, located in the Zhielieznaya and Volosianaya in mountain in Kandalaksha region, where there has been corrosion of these minerals as a result of amphibolization of rocks. Analised minerals with the highest coefficient derived from amphibolised granulite from the Sriednyj Myss Mt. of jagged shape. Low coefficient of fractal dimension occurs in after pyroxene garnets (xenomorphic, rounded crystals) present in metagabbro on the northern slopes of the northern peak Zhielieznaya and southern Zhielieznaya slope metavolcanites. The highest coefficient characterized by garnets from paraamphibolites of top of Zhielieznaya Mt., granules of paragranulites in Siennaya Kurtiazhnaya.

These minerals are very jagged, contain a large number of inclusions of quartz, titanite and rutile, have strongly expanded boundaries. The lowest coefficient characterized by after-pyroxene granoblasts of garnet in metagabbroids (of round-worms shape), exposing in amphibolites in the

northern parts of Kandalaksha region and within granulite from the surrounding mountains Sriednyj Myss [14].

Grains of quartz occurring in quartz veins cutting amphibolites have a slightly higher ratio of fractal dimension. They have extended the boundaries of a suture, resulting from the high growth accompany the processes of creating and participating vein at the same shear stress.

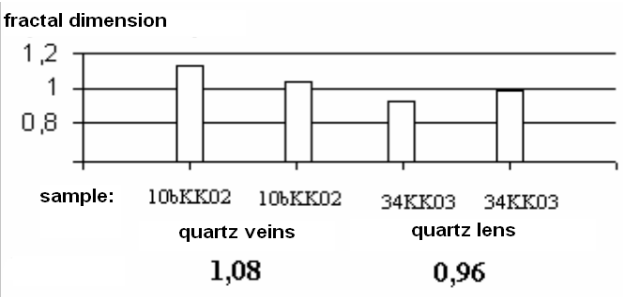


Fig. 17. The fractal dimension of the pieces of quartz core and lenses.

Table 1. Accesoring minerals in the studied rocks.

Type of rocks	Minerals
garnet amphibolite	rutile, tytanite, ilmenite, anatase, pyrite, chalcopyrite, magnetite, zircone
massive amhibolite	pyrite, titanite, chalcopyrite, rutile, ilmenite, zircone
amphibolite-garnet schists	titanite, rutile, apatite, ilmenite, monazite, cassiterite, phosphate, cerium and lanthanum
amphibolite-feldspar schists	titanite, rutile, ilmenite, pyrite, chalcopyrite, vanadium rutile
garnetites	rutile, ilmenite, tytanite, brookite,
metamyllonites	rutile, tytanite, ilmenite, pyrite, hematite, maghemite
pyroxene-garnets eclogites	rutile, brookite
metagabbroides	ilmenite, magnetite, tytanite, apatite, pyrite, chalkopyrite, zircone
para-granulites	ilmenite, rutile, spinels (chromite, hercynite), chromium garnets
basic-granulites	magnetite, hercynite, ilmenite, rutile, tytanite, pyrite, chalkopyrite, apatite, barite
charnockites & enderbites	pyrite, hematite, ilmenite, magnetite, rutile, chalkopyrite, zircone, fosphate of thor and cerium
veins rocks	pyrite (granitoids, ore veins), hematite and goethite (granitoids, ore veins), minerals lanthanides and cerium (prehnite-albite veins, tremolite-biotite veins), magnetite (ore veins)

4. DISCUSSION

Analyses of minerals from these metamorphic rocks (hornblende, plagioclase, biotite) indicate the formation of their conditions of 800-900°C and 11.5-12.5 kbar (the results obtained mainly for orthometamorphites). These are the conditions characteristic of the granulite facies. This is also indicated ferricity of hornblende F = 61% and the titanium content of biotite and ferricity F = 59% of metagabbros of Kandalaksha region (sample 22dK K02). Bogdanova et al. [29] and Mints et al. [33] state that around Kandalakssa is a multi-metamorphism (polimetamorfizm). The oldest stage (granulite facies) which is characterized by a pressure of 8-12 kbar and lower than indicated above temperature (740-850 ^{0}C). This difference may be related to the complex present in the ortho- and parametamorphites. After this phase, the Russian researchers stand-pressure metamorphism in amphibolite facies 8-12 kbar, 670-710^{0}C and 6-8 kbar and 600-650^{0}C. In the process of veins crystallization the prehnite-chloritie metamorphism was performed in surrounding rocks at the 2 kbar and 300^{0}C [14]. Granitoide and quartz veins formed at amphibole-epidote facies, and the prehnite-albite-calcite veins in prehnite-chlorite facies. In meta-morphosed igneous rocks are visible structure arrangement of melanocratic minerals in the rock. In metasediments marked a distinct directionality, underlined laying

amphibole needles and blades of biotite. In some rocks is visible the original fractional layering highlighted by increased gradation of minerals and participation of quartz. Few mineral parageneses primary preceding the highest degree of metamorphism. Their existence can only be inferred indirectly, based on the shape of the numerous detrital quart, and finally the most metamorphosed rocks under zonal zircone construction. In amphibolites is sometimes visible pseudo-eye structure distinguished by a greater focus garnets sometimes with rotation usually overgrown quartz "border". Similarly is visible the old plastic deformation probably sandy sediments in lens quartz, the presence of garnets gradual transition between the clusters and surrounding quartz minerals from amphibolite. Facies metamorphism of the highest degree, found in many of the studied rocks are granulite facies. It has developed both in the original rocks of the gabbro group as well as within the various sediments. The product of both the metamorphism are granulites. Some amphibolites derived from tectonic zones have characteristics indicating a high degree of transformation. Evidence of this is found in these minerals such as garnets rich in pyrope molecule and clino- and orthopyroxenes. In some cases, the (local) metamorphism was very strong, as evidenced by the occurrence hercynite in the rock and exceptionally high content of garnets, e.g. garnetites (more than 90% vol.), eclogites. In the case of amphibolite parageneses is represented by: rutile, diopside, high-titanium hornblende. In the veins rocks appear pyroxene (hipersten) and garnets (charnockitization of leucocratic rocks). The next phase is noted of amphibolitization of metamorphic rocks. Products in this facies are mainly due metamorphism of volcanics and gabbro-anorthozite intrusives. This process is reflected most strongly in granulites, which appears in the superseding hornblende pyroxene. The amphibolites are reflected in an increase in garnet richer in almandine molecule, the appearance of titanite (often around rutile). In this facies are also some myllonites. The rocks of these involved automorphic garnets recrystallized in crushed rock background. The rocks, especially in the vicinity of thrusts and veins, marked by a facies metamorphism developed in amphibol-epidote. It manifests itself occurrence of epidote. In some myllonites present epidote and chlorite. Sometimes they are also spindle-blasts of quartz, arranged perpendicular to the extension of vein quartz. The core pieces often occurs microcline, biotite, muscovite sometimes, rarely hornblende. In these rocks is also poorly marked facies metamorphism developed prehnite-chlorite. Evidence of this is the presence of metamorphic rocks albite, sericite, carbonates, chlorites and prehnite. The alkaline rocks appears saussuritization (there numerous calcite and albite). In the zone of weathering of these rocks there is a large amount of iron oxides (goethite, lepidocrocite) and growing smektitization of feldspar.

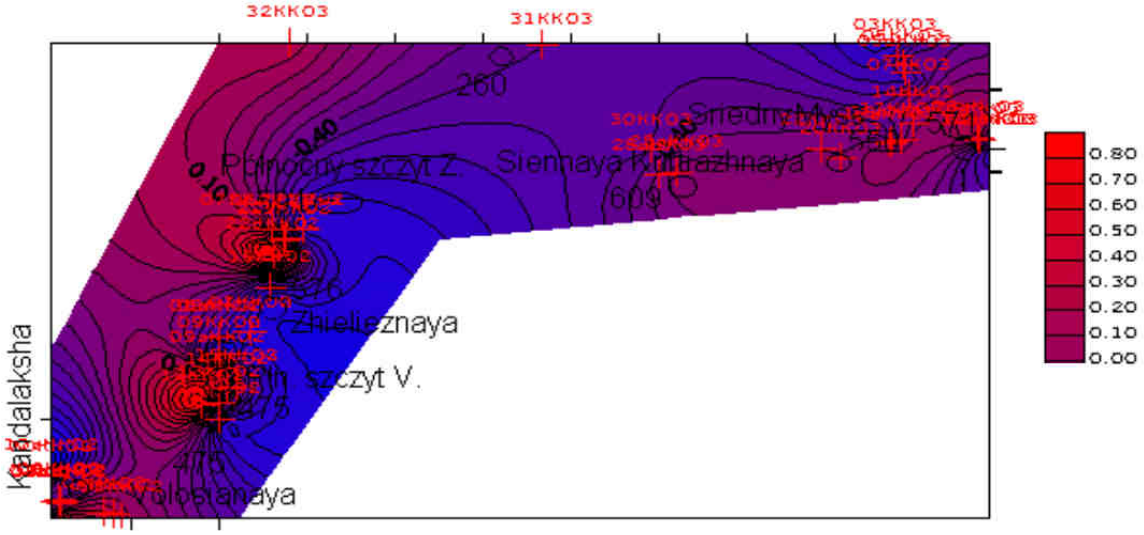
4. CONCLUSIONS

In conclusion it should be emphasized that these rocks have many changes both secondary and metamorphic that overlapping rubbing contributed parageneses older. After a period of formation of the protolith, the rocks have been metamorphosed and then lifted as a block. During the process lift into certain zones created myllonites. During this time, the rocks have been metamorphosed to amphibolite facies conditions. After the end of tectonic activity in the Kandalaksha region have been numerous hydrothermal processes that contributed to the silification and enrichment of epidote, chlorite and desilification and enrichment of prehnite, albite, carbonates [14, 31]. Much led to modifications occurring rock in this area. The carbonating processes make in the formation of new structures and textures. Recreate all the changes taking place in these rocks, as well as an estimate of the scale and position in time, required the use of a number of different methods of research and critical approach to the obtained results, which are often a superposition of different information.

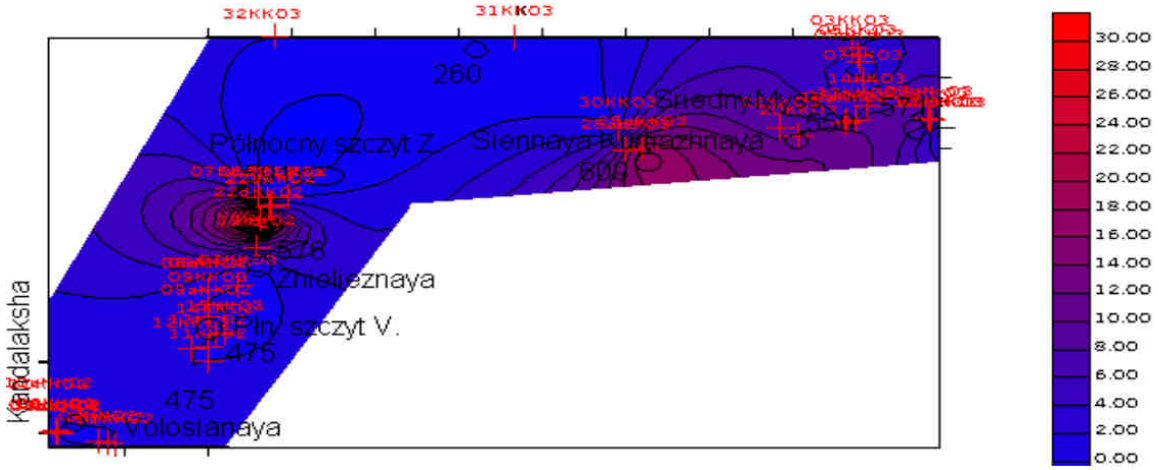
TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

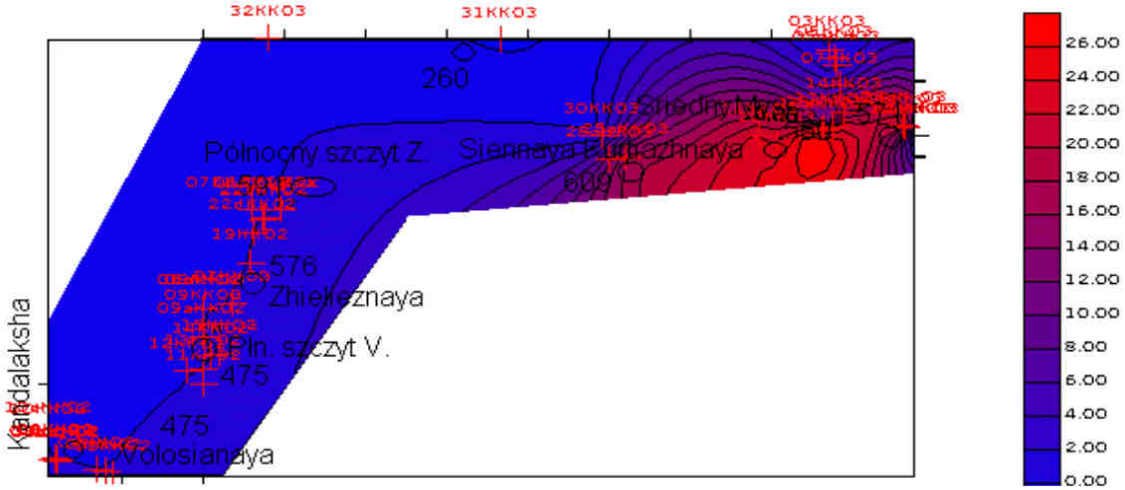
MAP ATLAS



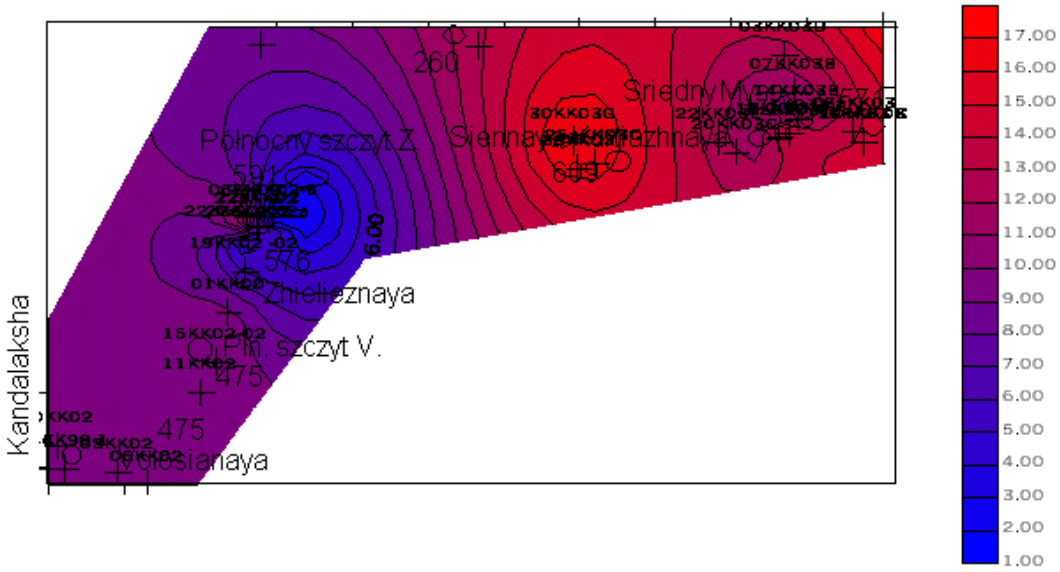
Map 1. Distribution indicator color in the studied rocks in the area.



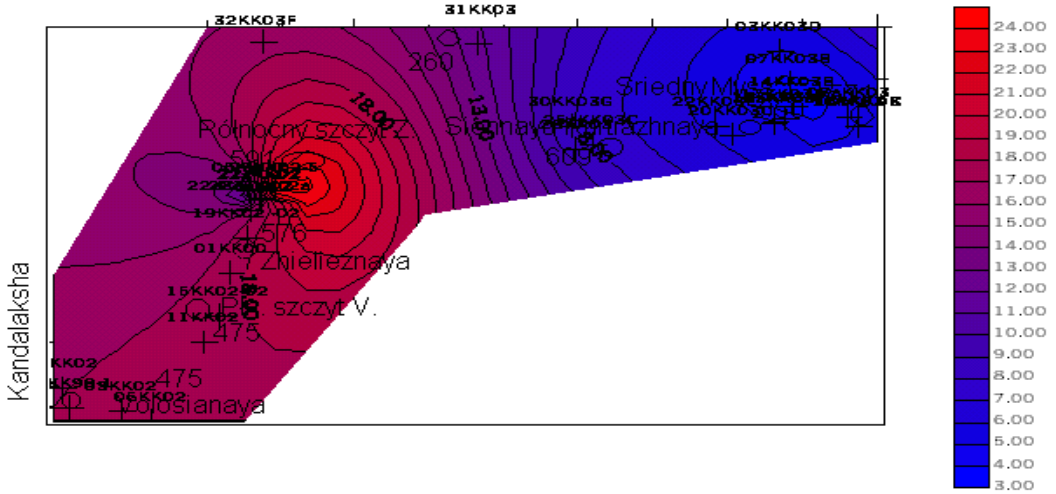
Map 2. Occurrence of clinopyroxene in the studied rocks field in vol %.



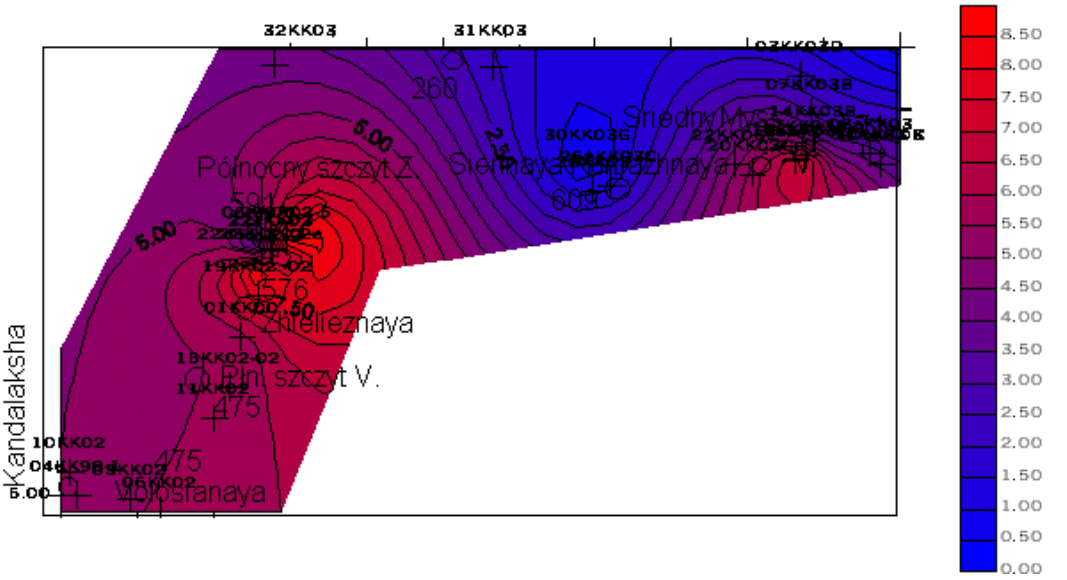
Map 3. Occurrence of orthopyroxene in the studied rocks field in vol %.



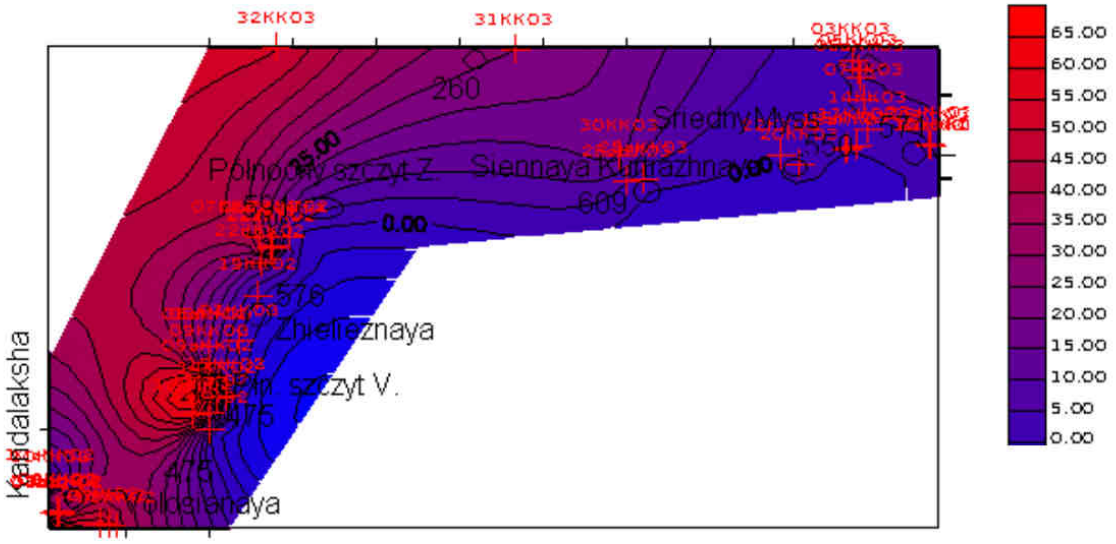
Map 4. Distribution of Ca in the pyroxenes [wt %].



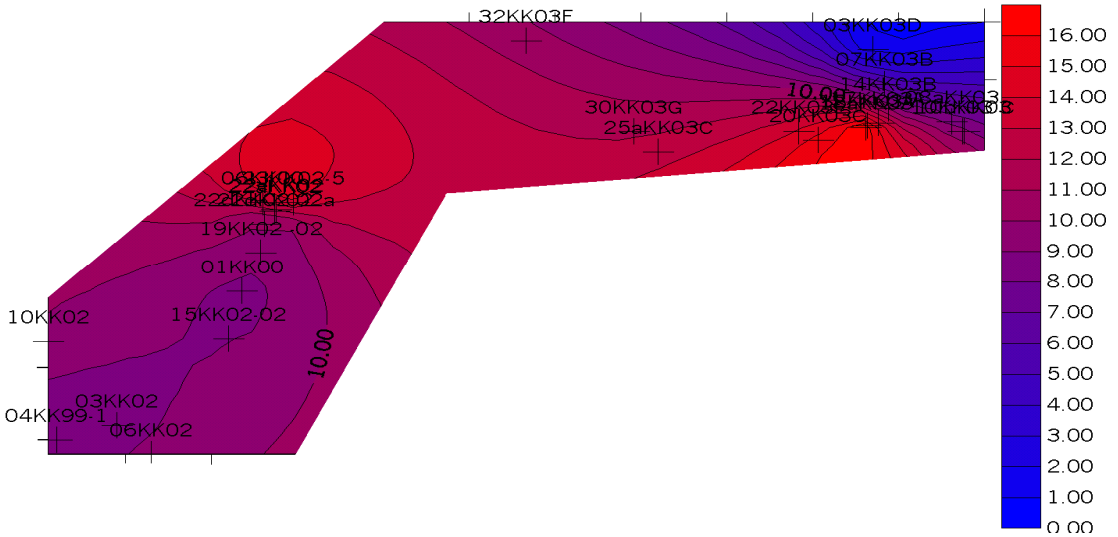
Map 5. Distribution of Fe in the pyroxenes [wt %].



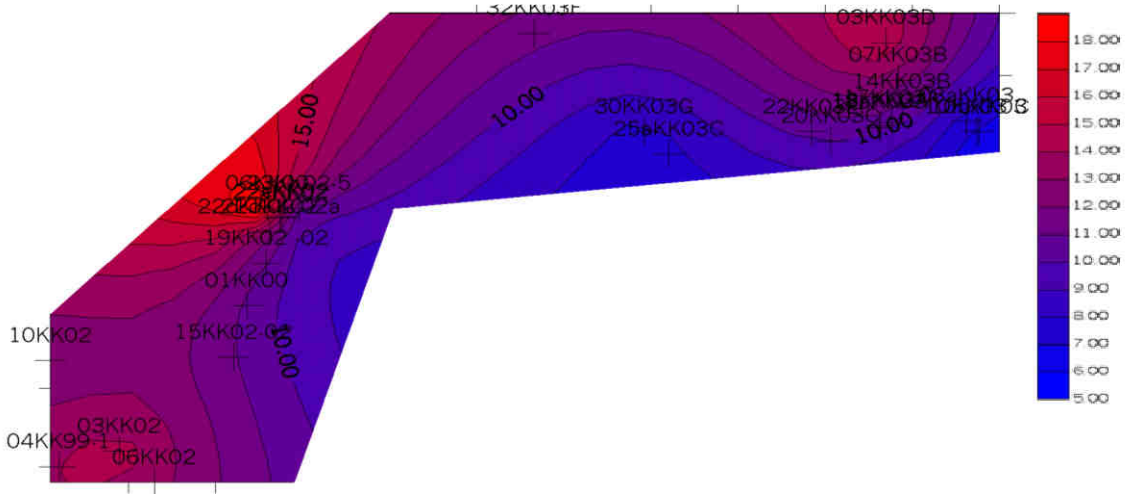
Map 6. Distribution of Mg in the pyroxenes [wt %].



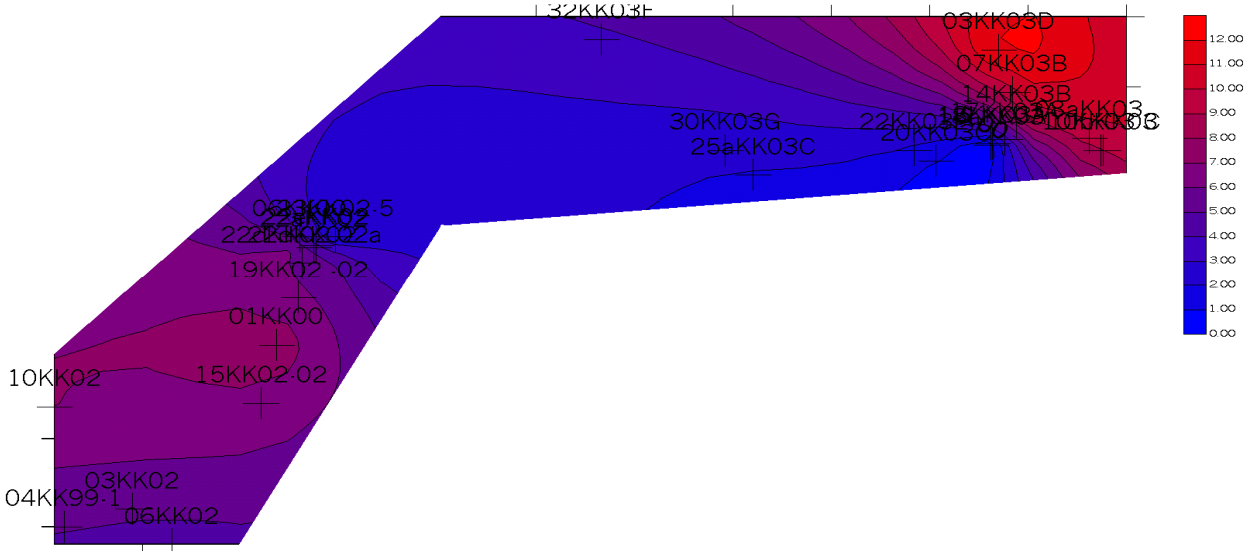
Map 7. Distribution of ampfiboles in the stury area [vol %].



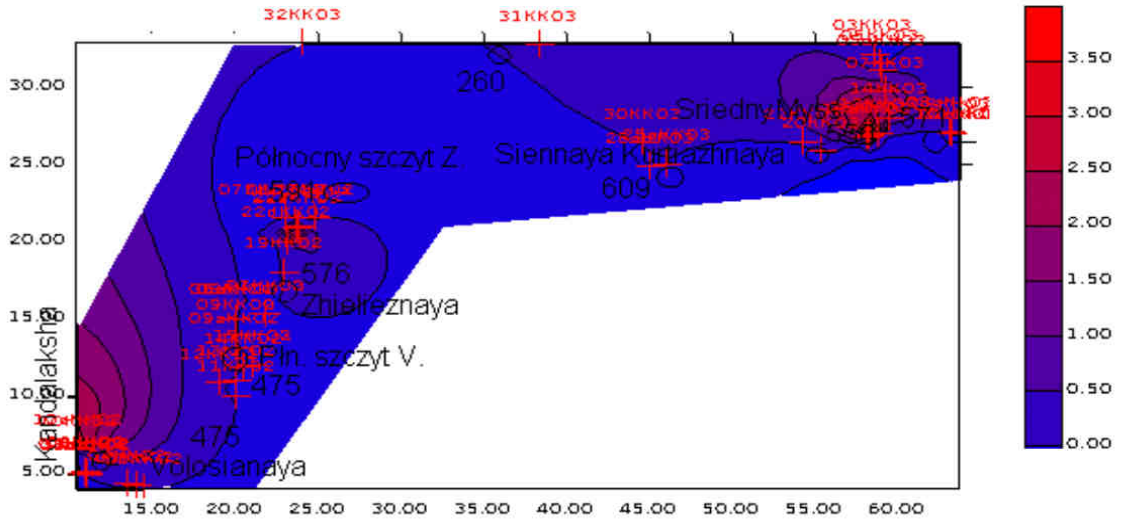
Map 8. Distribution of Ca in the amphiboles [wt %].



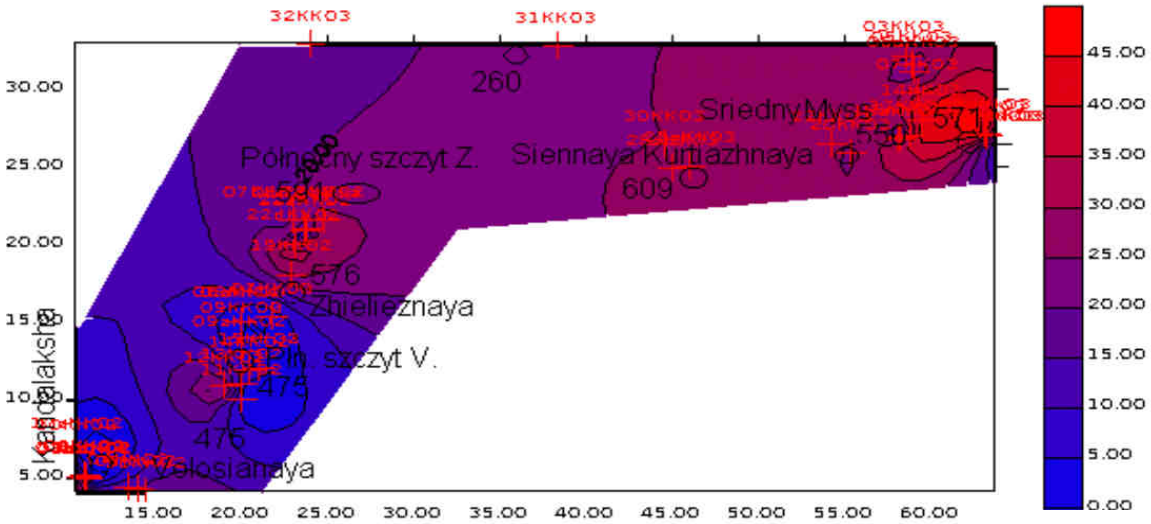
Map 9. Distribution of Fe in the amphiboles [wt %].



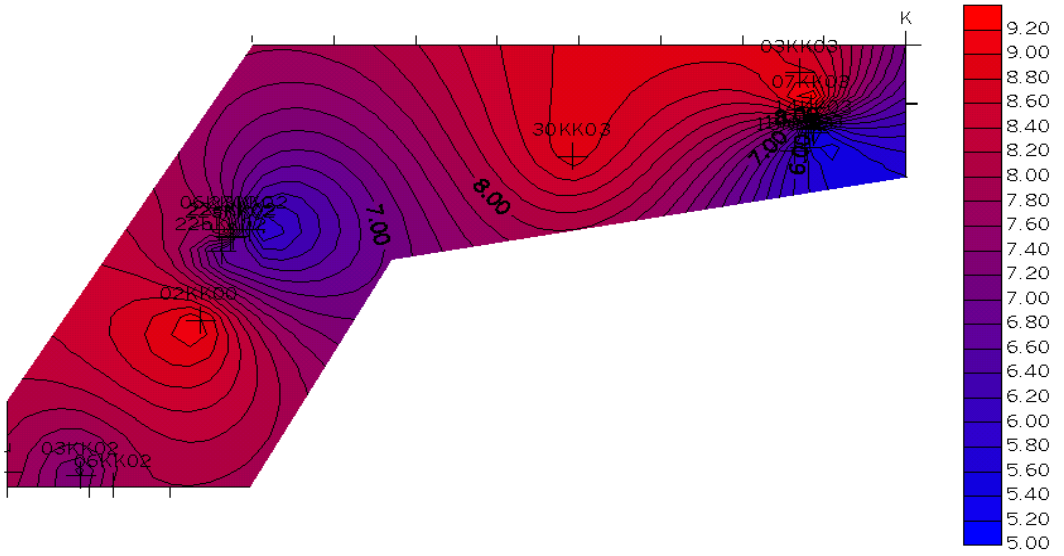
Map 10. Distribution of Mg in the amphiboles [wt %].



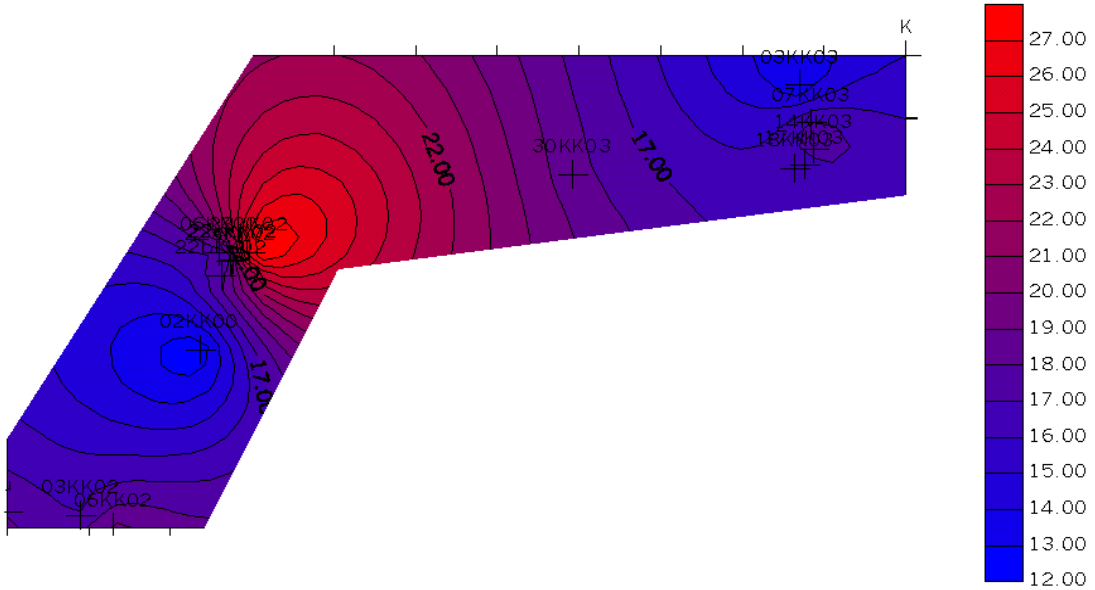
Map 11. Distribution of biotite in the study area [vol %].



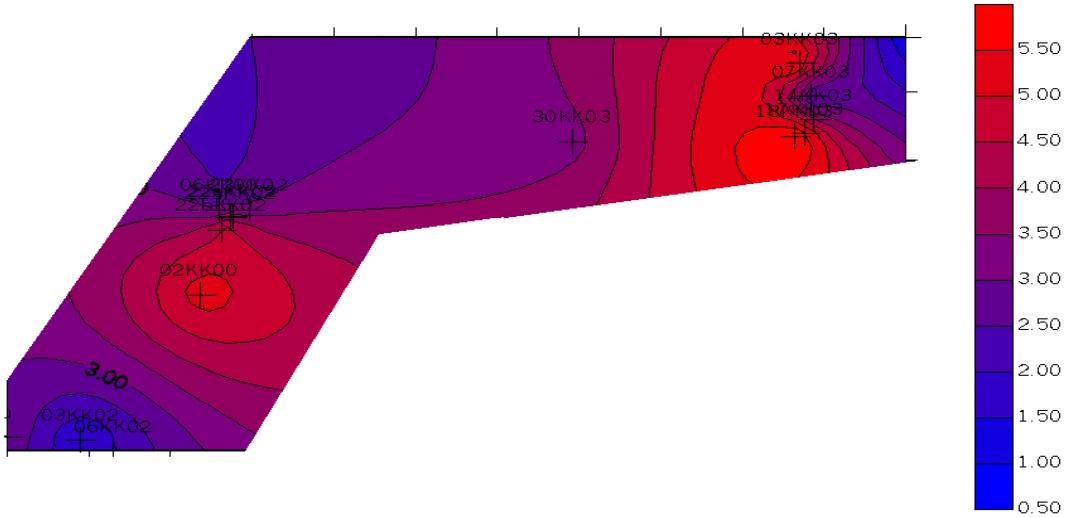
Map 12. Distribution of garnet in the study area [vol %].



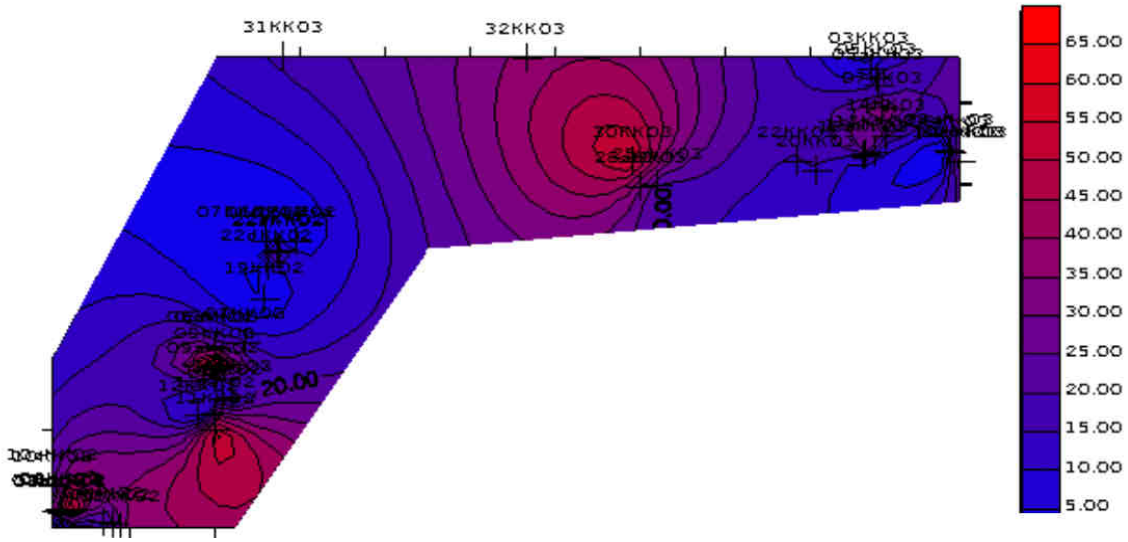
Map 13. Distribution of Ca in garnets [wt %].



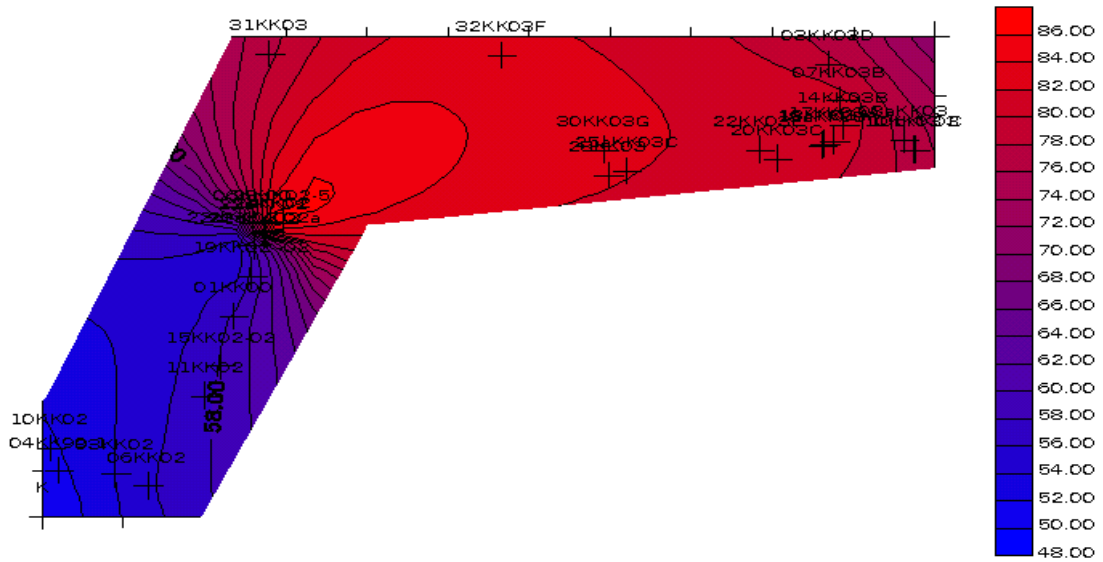
Map 14. Distribution of Fe in the garnets [wt %].



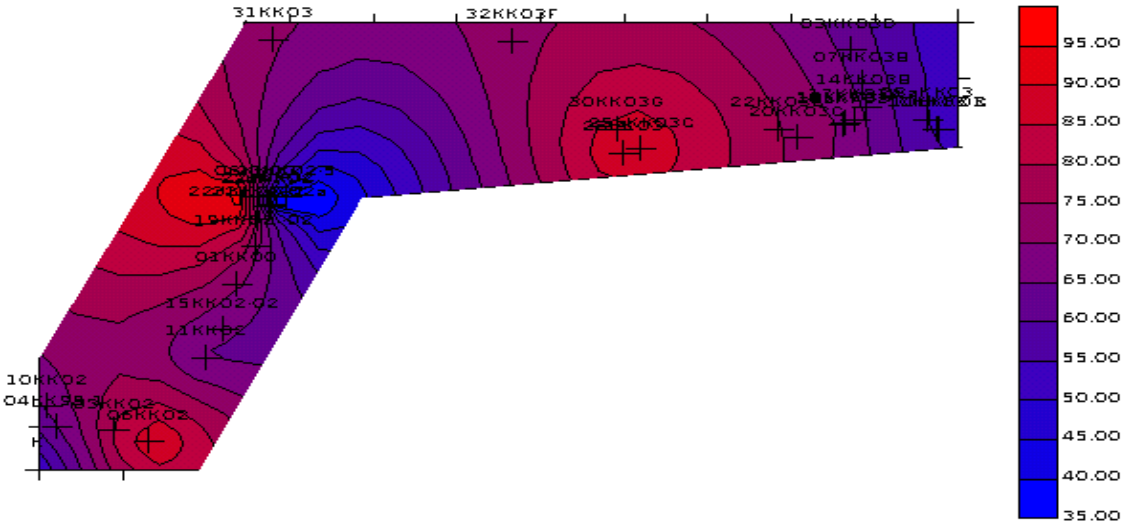
Map 15. Distribution of Mg in the garnets [wt %].



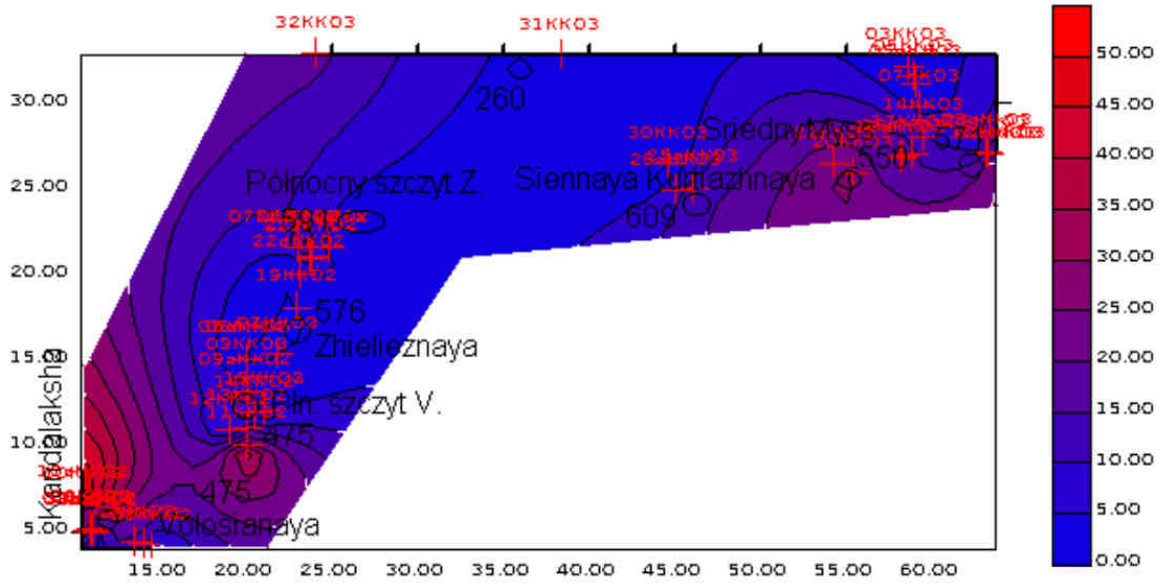
Map 16. Distribution of feldspars the study area [vol %].



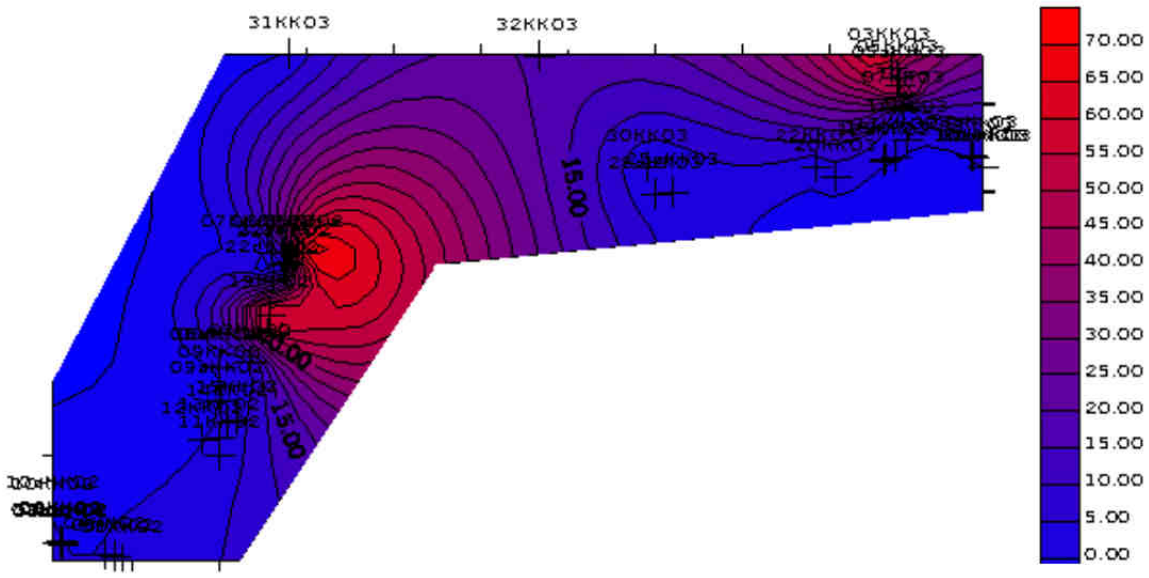
Map 17. Type of basic plagioclase (metamorphic) and their distribution in the study area.



Map 18. Type of acid plagioclase (secoundary type) and their distribution in the study area.



Map 19. Distribution of quartz the study area [vol %].



Map 20. Distribution of the chlorites, epidotes and ore minerals in study area [vol %].

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