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Biopesticidal Activity of Lichenic Extracts against the Grain Storage Pests *Tribolium castaneum* and *Sitophilus oryzae*

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A B S T R A C T

Insects cause great damage to agricultural crops. A secondary effects of pest is viral, bacterial and fungal infection where are facilitated by the damages and injured to crop plants by insects and mites. The yield loss of crops by nibbling, injury and growth retardation is estimated to be 20% - 30%. An additional 10% is lost during post harvest storage and transportation. Decrease in crop quality causes further economic loss.

Introduction

Pesticides are active chemicals used for killing plants or animal pests. It is a general term embracing insecticides, herbicides, fungicides nematocides etc., Pesticides may have dual action. They are important in controlling injurious pests, but they may also present a hazard to species not considered to the pest in the environment. Pesticides enter adequate environment through aerial drift or run off from applications or accidental release and it become rapidly disturbed through the action of wind and water .Agricultural runoff from field and grazing land is considered as the major route of pesticide movement into water (Li, 1975). Improper use of the pesticides cause reduced shell growth in oyster (Parrish *et al.*, 1976) feeding activity in lungworm, respiration in yellow perch where as the biopesticides do not cause any side effects. Botanical insecticides are naturally occurring insecticides that are derived from plants (Isman, 2000). The insecticidal activity of essential oils and plant extracts against different stored-product pests has been evaluated (Shaaya *et al.*, 1991; Sarac and Tunc, 1995; Tunc *et al.*, 2000; Kim *et al.*, 2003; Lee *et al.*, 2003; Aslan *et al.*, 2005; Cetin and Yanikoglu, 2006; Negahban *et al.*, 2007, Ayvaz *et al.*, 2009). In spite of the wide-spread recognition that many plants possess insecticidal properties, only a handful of pest control products directly obtained from plants

are in use because the commercialization of new botanicals can be hindered by a number of issues (Isman, 1997). Botanicals used as insecticides presently constitute 1% of the world insecticide market (Rozman *et al.*, 2007). Essential oils from different plant species possess ovicidal, larvicidal, and repellent properties against various insect species and are regarded as environmentally compatible pesticides (Isman, 2000; Cetin *et al.*, 2004). Hence this study is aimed to explore the biopesticidal properties of selected lichens against the devastating, grain storage pest, *Tribolium castaneum* and *Sitophilus oryzae* under laboratory conditions.

Materials and Methods

Collection and Maintenance of Storage Pests

The pests (prey) under study were collected from the Grain storage area. The eggs and pupae of the red flour beetle, *T. castaneum* were maintained in the controlled from temperature room at 30^{o}C. *Tribolium castaneum* eggs, small (1st instars) larvae, large (4th and 5th instars) larvae and pupae whereas small (1st and 2nd instars) larvae and large (4th instars) larvae of *Sitophilus oryzae* were used in the experiment. *T. castaneum and Sitophilus oryzae* eggs were obtained by releasing 50-100 adults in glass jars (250 mL) containing flour, which is shifted daily through 70 size mesh sieves.

First and second instars larvae of all these pest species were obtained after hatching from their eggs held in petri dishes containing a small amount of wheat flour for 3-7 days. Fourth and fifth instars larvae and pupae were collected from the petridishes after elapsing the respective developmental stages. Larvae and pupae were collected from the petri dishes by sieving with a 70 mesh sieves and counted carefully by a fine brush.

Collection of lichens

Different species of lichens *viz.*, *Usnea cf.nilgirica* G. Awasthi (Parmeliaceae), *Usnea undulata* stirton (Parmeliaceae), *Usnea maculata* stirton (Parmeliaceae), *Ramalina conduplicans* Vainio (Family: Ramalinaceae), *Rocella montagnei* Bel.em.D.D. Awasthi (Rocellaceae)were collected and authenticated. The collected sampleswere washed thrice with tap water and twice with distilled water to remove the adhering associated animals. A sample voucher specimen was deposited in the herbarium facility.

Extraction of bioactive Principles

The samples were cut into pieces and kept for shade drying. After the complete removal of moisture content the samples were subjected for percolation by soaking in organic solvents with different polarity *viz.*, ethanol, ethyl acetate, methanol, acetone, chloroform. After 21 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (>45°C) and then freeze dried (-80°C) to obtain solid residue. All the extracts were dissolved in dimethyl sulphoxide (Hi media Laboratories Private Limited, Mumbai, India) and filtered through sterile millipore filters (mesh 0.20 µm, Sartorious Stedim Biotech GmbH, Germany).

Preliminary screening – Artemia toxicity assay

Healthy adults of *Artemia parthenogenetica* were used for this bioassay. Brine shrimp lethality assay is a convenient and rapid assay for the detection of bioactive compounds for pesticidal activity. Research has shown that *Artemia* toxicity assay correlates well with anti-tumor activity. Fifty milliliter of filtered sterilized and cold sea water was taken in a small bowl and 10 numbers of *Artemia*

adults were introduced into it. This bowl was treated as control. Lichenic extract of different concentrations (500 $mg.ml^{-1}$, 50 $mg.ml^{-1}$, 25 $mg.ml^{-1}$, 10 $mg.ml^{-1}$) were added into a total volume of 50 ml in all the bowls. Ten numbers of artemia adults were introduced into each bowl and were treated as experimental. Control was also maintained with the addition of lichenic extracts. Triplicates were maintained for each treatment and the average values were taken for comparison. Mortality of *Artemia* was observed after 24 hours. Mortality in the experimental was compared with that in control. Percentage of mortality was calculated by the following formula:

Mortality (%) = No. of Artemia died/No. of Artemia treatedx100

Biopesticidal activity of crude seaweed extracts against chosen grain storage pests (Secondary Screening)

A set of ten healthy insects of *T. castaneum* and *S. oryzae* were introduced in to a series of sterilized petriplates. One drop of the most potent lichenic extracts which showed maximum artemia mortality was prepared with methanol was spotted on each insect. Another set of ten healthy insects of each species were introduced separately into 2 dried and sterilized petriplates. One drop of methanol, the organic solvent alone was spotted on each insect and maintained as control. Then the plates were covered and kept undisturbed for 24 hours. After 24 hours, the plates were observed for survivors. Mortality results were quantified as petriplates with no survivors. Mortality in the plates with extract applied insect was compared with the control plates. Percentage of survival and mortality was calculated by the following formula: Survival (%)= No. of survivors/Total number of insects trreatedx100 and Mortality (%) = No. of insects died/No. of insects treatedx100

To determine the polarity of the active compounds, partitioning was carried out. The extracts were partitioned fist between ethyl acetate and the water phase is subsequently partitioned against n – butanol. Non-polar compound were partitioned into the ethyl acetate whereas compounds of intermediate polarity were partitioned into the n-butanol. Water soluble metabolites remained in the aqueous phase. Partitioning was carried out by using a separating funnel. The phases were separated and evaporated for concentration. After concentration, the phases were tested for insecticidal activity.

Bioassay guided fractionation of the most effective lichenic extracts

Chromatography is typically the next step after determination of the polarity of the extract components. It is done to separate the different active compounds in the extracts. The most effective lichenic extracts exhibiting potent insecticidal activity were fractioned using silica gel chromatography. The column was eluted successively using, ethyl acetate and methanol in different proportions and the fractions were collected separately. Then each fraction was tested for insecticidal activity. The different proportions of methanol, ethyl acetate and methanol used for fractionation are -methanol, 1:3 (v/v) – Ethylacetate: methanol, 1:1 (v/v) – Ethylacetate: methanol, 3:1 (v/v) - Ethylacetate: methanol, 50- Ethylacetate: methanol, 1:1 (v/v) - Ethylacetate: methanol.

Biopesticidal activity of fractionated compounds against grains storage pests

A set of ten healthy insects of *T. castaneum* was introduced in to a series of sterilized petriplates. One drop of fractioned bioactive compounds from lichens was spotted on each insect. Another set of ten healthy insects of each species were introduced separately into 2 sterilized petriplates. One drop of methanol,

the organic solvent alone was spotted on each insect and maintained as control. Then the plates were covered and kept undisturbed for 24 hours. After 24 hours, the plates were observed for survivors. Mortality was observed if no survivors and compared with the control. Percentage of survival and mortality was calculated.

Results and Discussion

Artemia parthenogenetica is one of the most, if not the most, convenient organism for toxicity tests on microscopical invertebrates. The literature available as well as the results given above demonstrates, however, that a number of precautions must be taken with regard to the reproducibility of the results. Factors which should be kept under strict control are the exact origin of the strain, the temperature during incubation and hatching, the moment of harvesting of the larvae, the period of time between the harvest and the start of the bioassay, and the temperature and salinity of the medium during the test. Considering the changes in sensitivity of larvae of different morphological development and also considering the fact that any test carried out with larvae from the third larval stage will imply feeding the organisms prior to or during the test, the present results suggested that bioassays with *Artemia* larvae be carried out only as short term toxicity test and only on freshly hatched nauplii.

Healthy adults of *Artemia parthenogenetica* were used for this bioassay. Brine shrimp lethality assay is a convenient and rapid assay for the detection of bioactive compounds for pesticidal activity. Research has shown that *Artemia* toxicity assay correlates well with anti-tumor activity. The obtained results are shown in Table 1.

The selected lichens were treated as pesticidal activity against storage pest *Tribolium castaneum* and *Sitophilus oryzae* at concentration of 50 $mg.ml^{-1}$. The percentage of mortality of storage pests were tabulated (Table 2). Evaluation of natural products and synthetic compounds by using brine shrimp cytotoxicity assay describes not only cytotoxicity but also anticancer, antiviral, insecticidal and pesticidal potential (Sheikh *et al.*, 2004). A good correlation has been found between brine shrimp cytotoxicity and cytotoxicity against KB cells (McLaughlin, 1991). Awal *et al.*, (2004) has demonstrated toxicity of leaf and seed extracts of *Cassia alata* by using brine shrimp cytotoxicity assay while in another study by Mongelli *et al.*, (2003), cytotoxic evaluation of components of *Bolax gummifera* was demonstrated by using brine shrimp cytotoxicity assay. Brine shrimp assay has also been used by Chowdhury *et al.*, (2004) while describing cytotoxic potential of extracts and purified components of *Stachytarpheta urticaefolia*.

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they have fumigant activity that might be of importance for controlling stored product insects (Konstantopoulou *et al.*, 1992; Regnault Roger and Hamraoui, 1995; Ahn *et al.*, 1998).

In the current study, the bioactive compounds obtained from lichens were showed insecticidal activity against the adults of *Tribolium castaneum* and *Sitophilus oryzae*. Globally, there is a growing awareness and desire to utilize natural and ecofriently compounds for insect pest control. In this connection, essential oils, plant extracts and phytochemicals which are complex mixtures of individual compounds have been investigated extensively which possesses broad spectrum of pest control properties. The botanical extracts from the plant leaves, roots, seeds, flowers and basic in their crude form has been used as conventional insecticides

from centuries. One plant species may possess activities with a wide range, for example extracts from the neem tree are antifeedant, oviposition deterrent, repellent and growth regulating (Schmutterrer, 1995; Raguraman, 1997). Effect of seed extract on the fecundity and fertility of *Tribolium castaneum* and *T. confusum* of *J. gossypifolia* were reported (Khanam *et al.,* 2008). The MeOH extracts of *J. gossypifolia* were assayed for their toxicity against the early fourth instar larvae of *C. quinquefasciatus* (Rahuman *et al.,* 2008).

In the last three decades many plant allele-chemicals including Phyrethrum, nicotine, azadirachtin and rotenone were isolated, characterized and developed as effective pest control agents (Gonzalo, 2004). The secondary compounds of plants are a vast repository of compounds with wide range of biological activities. Impact of phenolic compounds on the larvicidal activity has been reported previously (Tripathi and Rathore, 2001). There are meager reports on extract or compounds evaluation against non target organism to ensure its safety in environment. Saidaina *et al.,* (2007) reported sixteen halophytic plant extracts for their bio-insecticidal activities against larvae and adults of *Tribolium confusum*. Anti-feedent effect, toxicity and insect growth inhibition were followed up *Frankenia laevis, Suaeda echioides* and *Tamarix boveana* ethyl acetate extracts were more powerful inhibitor of feeding than the others. They presented high toxicity and affected significant larval growth of the confused flour beetle, when applied at a concentration of 1%.

The present study also made an attempt to findout the pesticidal property of different fractions by using the ethyl acetate and methanol at different proportions and represented in tables 3-7. It reveals that, the lichenic species of *Usnea maculata* showed 100% insecticidal property against the storage pest *Tribolium castenum.*

Our findings are in accordance with the findings of Mahfuz and Khanam (2007), who reported that petroleum ether extract of *D. stromonium, Corchorus capsularis*, *Aphanamixis polystachea* and *Jatropha curcas* exhibited piquant toxic effect against *T. confusum* Duval adults. The recent results is in conformity with the result of Khanam *et al.,* (2009) who reported that petroleum ether, acetone and methanol extract of *Trichosanthes palmate* seed showed strong toxic effect to the larvae and adults of *T. castaneum* and *T. Confusum.* Kim *et al.,* (2003) revealed the significant insecticidal activity of *Cinnamonum cassia* bark, oil, *Cocholeria aroracia* oil and *Brassica juncea* oil within 24 hours after treatment against adults of *Sitophilus oryzae* and *C. chinensis* using direct contact application methods. Arabi (2008) reported that, the oil of *Provskia abrotanoides* karel at 322 µl.l^{-1} air caused 100 %mortality is *S. oryzae and T. castaneum* within 13 and 7 exposure time respectively. He also reported that, mortality of *S. oryzae* and *T. castaneum* increases with the increases of the exposure time (2 to 15 hours duration) at all the concentration (32, 161, 322, 483, and 645 µl. l^{-1}). Sahaf *et al.,* (2007) revealed the significant insecticidal activity of *Carum copticum* essential oil against *S. oryzae* and *T. castaneum* adults. Result of the present study is in agreement with the results of Park *et al.,* (2002), who reported that, essential oil of *A. calamus* oil was toxic to *S. oryzae* and *C. chinensis* adults when applied tropically. Wanyika *et al.,* (2009) reported that, natural pyrethuum extract blended with cotton seed oil exhibited the highest mortality against the adult maize weevils, *Sitophilus ozeamais* Motschulsky.

*Sargassum*brown seaweed found along the coast of Japan, China, India, Pakistan and

Spain. *Sargassum boveanum* and *S. ilicifolicem* possess insecticidal activity against *Collobeuchus analis* and *Trigoderma grannarium* (Rizvi, 2003). Razvi and Shameel (2004) later reported the insecticidal activity of benthic algae belonging to the Chlorophyta, Phaeophyta and Rhodophyta. The present study clearly revealed that, the *Sargassum wightii* had potential insecticidal activity. The presence of phenolics, alkaloids, favanoids, steroids and saponins in the crude extracts of *Sargassum wightii* suggest that, seaweeds can be used as antimicrobial, anti-parasitic, anti-inflammatory, anti-feedant, antioxidant, anti-allerginic, anti-thrombic, anti-carcinogenic and anti- ulcers agents in the near future (Johnson Marimuthu *et al.,* 2012).

Similar to higher plants, lichens are considered as potential of novel biologically active compounds. Lichens are complex symbiotic associations between a fungus (mycobiont) and an alga (photobiont) with unique characteristics in plant kingdom. It is estimated that there are approximately 25,000 species of lichens (C h a p m a n, 2004). They are proven as the earliest colonizers of terrestrial habitats on the earth with a worldwide distribution from artic to tropical regions and from the plains to the highest mountains (Taylor *et al.*, 1995). Specific, even extreme, conditions of their existence, slow growth and long duration (age of several thousand years) are result of numerous protective compounds against different physical and biological influences (Denton and Karlen, 1973). The desert species *Lecanora esculenta* is considered as "biblical manna" (Trease & Evans, 1978). It is known that Romans dyed their togas with orchil, a purple pigment from *Roccella sp.* and crottal, brown pigment from *Parmelia, Ochrolechia* and *Evernia sp.* (Muggia *et al.,* 2009). Lichens dyed textile reached considerable economic importance in 18th century in some parts of the world as in the Canary Islands (Muggia *et al.,* 2009). Litmus, a blue coloring matter from lichen fermentation, was used as dye for textile and beverages (B e e c k e n *et al.,* 1961). Extracts of some species of lichens, like *Evernia prunastri*, are contents of perfumes (Trease and Evans, 1978). Naturally, the most important and studied application of lichens is the one in traditional medicine for treatment of animals and humans. For instance, New Zealand Maori traditionally use long, pendulous species of *Usnea* for nappies and sanitary pads (Perry *et al*., 1999). Also, *Usnea* species have been used in Asia, Africa and Europe for pain relief and fever control (O k u y a m a *et al.,* 1995). *Usnea densirostra*, known as "barba de la piedra" served as a cure for various disorders in Argentina's folk medicine (Correchea *et al*, 1998). Two lichen species, *Parmelia caperata* and *Umbilicaria sp.* are reported in study of Chilean traditional medicine (Munoz *et al.,* 1981). *Ramalina thrausta* is used in Finland for treatment of wounds, athlete's foot or other skin diseases and taken to relieve sore throat and toothache. *Cetraria islandica* is ancient cough remedy known as *"tonicum amarum"* accepted as a mucilage drug (Muller, 2001). Various species worldwide are used in traditional medicine and said that they are able to cure dyspepsia, bleeding piles, diabetes, bronchitis, pulmonary tuberculosis, spermatorrhoea and other diseases of the blood and heart (Richardson, 1988). Generally, lichens metabolites can be divided into two groups: primary and secondary. Primary metabolites are proteins, lipids, carbohydrates and other organic compounds involved in lichen's metabolism and structure. Secondary metabolites, also known as lichens substances, are produced mainly by the fungus and secreted onto the surface of the lichen's hyphae either in amorphous forms or as crystals. Secondary metabolites from lichens are complex, but predominantly small molecules, which comprise up to 20% of lichen's dry weight (Muggia *et al.,* 2009). Structures of more than

1000 different lichen substances are determined to date and many of them are pharmaceutically relevant (Muggia *et al.,* 2009). Secondary metabolites are products of polyketide pathway, mainly monocyclic and/or bicyclic phenols joined by an ester bond (depsides), both ester and ether bonds (depsidones) or furan heterocycle (dibenzofurans and usnic acid), antraquinones, xanthones, chromones and secondary aliphatic acids and esters (Stojanović *et al.,* 2011). Some of them are produced by the fungus or the alga, while others are exclusively produced by synergistic action of both partners in lichens. Whole spectrum of lichen metabolites evolved for protective purposes against various physical and biological environmental factors (Denton and Karlen, 1973). Large amounts of phenolic compounds fungal melanins are synthesized and accumulated in the tallus in order to absorb UVB light and shelter the photobiont from excessive radiation (G a u s l a a & S o l h a u g , 2001). These photoprotectors have great antioxidant capacity (Hidalgo *et al.,* 1994, Fernandez *et al.,* 1996) and can be used as preservatives in cosmetic products. Certain phenolic compounds protect lichens from herbivores (Lawrey, 1989). Other lichen metabolites have antibiotic properties which prevent microbial degradation of the thallus (Emmerich *et al.,* 1993). Some of lichen metabolites are involved in maintaining of the symbiotic equilibrium (Huneck, 1999), while others dissolved rocks for better attachment of lichens (Seaward, 1997). Biological activity of lichens and their metabolites exert manifold biological activity. First, antibiotic properties of lichen extracts are known for decades. First study by Burkholder originated from 1944. Later, it was reported antimicrobial activity of several lichen species. According to wide screening of antimicrobial activity of lichen extracts, it seems that bacterial inhibitions can vary within the lichen extract, solvent used for extraction and bacteria tested. Rankovic *et al.,* (2007a; 2007b) tested aqueous, acetone and methanol extracts of *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogimnia physodes, Umbilicaria polyphylla, Lasallia pustulata, Parmelia sulcata, Umbilicaria crustulosa and Umbilicaria cylindrica* from Serbia on six species of bacteria and ten species of fungi. The strongest activity was observed with methanol extracts of *Parmelia pertusa* and *Parmelia sulcata* and the weakest activity was manifested by *Parmelia caperata* and Umbilicaria cylindrical. Aqueous extracts of all tested lichen species were inactive. *Bacillus mycoides* was the most sensitive bacterial species tested, whereas *Candida albicans* was the most sensitive fungal species examined. Behera *et al.,* (2005) reported that acetone, methanol and light petroleum extracts of lichen *Usnea ghattensis* were effective against *Bacillus licheniformis, B. megaterium, B. subtilis and S. aureus.* Also, Karagoz *et al.,* (2009) evaluated aqueous and ethanol extracts of 11 different species from Turkey and determined potent antibacterial activity of aqueous extract of *Peltigera polydactyla* and ethanol extract of *Ramalina farinacea*. Recently, Mitrovi ć *et al.,* (2011) studied antibacterial activity of methanol extracts of five lichen species (*Flavoparmelia caperata, Evernia prunastri, Hypogymnia physodes* and *Cladonia foliacea*). Two lichen species were tested for the first time (*Evernia prunastri* and *Cladonia foliacea*). The analysis of their antibacterial potential were performed on 15 strains of bacteria and revealed the strongest inhibitory effect, especially on Gram (+) bacteria, of *Hypogymnia physodes* and *Cladonia foliacea.* Second, antifungal activities of certain lichen substances are also revealed. Manojlovic *et al.,* (2005) reported antifungal activity of the anthraquinone parietin isolated from *Caloplaca cerina*.

Table.1 Primary screening of lichenic extracts on the toxicity of *Artemia*

Solvent	Name of the species	Percentage of mortality			
		500 mg .ml-1	***50 mg. ml-1***	***25 mg .ml-1***	***10 mg. ml-1***
Ethanol	*Usnea nilgirica*	100	60	50	20
Ethanol	*Usnea undulata*	100	60	40	20
Ethanol	*Usnea maculata*	100	80	50	30
Ethanol	*Ramalina conduplicans*	100	60	60	20
Ethanol	*Rocella montagnei*	100	60	60	10
Ethyl acetate	*Usnea nilgirica*	80	80	60	40
Ethyl acetate	*Usnea undulata*	100	50	40	0
Ethyl acetate	*Usnea maculata*	100	60	50	30
Ethyl acetate	*Ramalina conduplicans*	100	90	60	60
Ethyl acetate	*Rocella montagnei*	70	60	40	20
Chloroform	*Usnea nilgirica*	80	50	20	0
Chloroform	*Usnea undulata*	80	70	60	40
Chloroform	*Usnea maculata*	90	50	40	20
Chloroform	*Ramalina conduplicans*	100	100	60	60
Chloroform	*Rocella montagnei*	100	40	30	0
Acetone	*Usnea nilgirica*	100	40	20	10
Acetone	*Usnea undulata*	70	60	0	0
Acetone	*Usnea maculata*	100	70	50	30
Acetone	*Ramalina conduplicans*	100	70	50	30
Acetone	*Rocella montagnei*	100	80	60	50
Methanol	*Usnea nilgirica*	20	0	0	0
Methanol	*Usnea undulata*	100	50	30	0
Methanol	*Usnea maculata*	90	50	40	0
Methanol	*Ramalina conduplicans*	100	70	50	20
Methanol	*Rocella montagnei*	100	100	50	20

Table.2 Secondary screening of lichenic extracts on the toxicity of storage pests

Solvent	Name of the species	% of mortality	
		Tribolium castaneum	***S. oryzae***
Ethanol	*Usnea nilgirica*	100	100
Ethanol	*Usnea undulata*	100	100
Ethanol	*Usnea maculata*	100	100
Ethanol	*Ramalina conduplicans*	100	100
Ethanol	*Rocella montagnei*	100	100
Ethyl acetate	*Usnea nilgirica*	100	100
Ethyl acetate	*Usnea undulata*	100	100
Ethyl acetate	*Usnea maculata*	100	100
Ethyl acetate	*Ramalina conduplicans*	100	10
Ethyl acetate	*Rocella montagnei*	100	100
Chloroform	*Usnea nilgirica*	100	80
Chloroform	*Usnea undulata*	100	100
Chloroform	*Usnea maculata*	100	100
Chloroform	*Ramalina conduplicans*	100	100
Chloroform	*Rocella montagnei*	100	100
Acetone	*Usnea nilgirica*	100	100
Acetone	*Usnea undulata*	100	100
Acetone	*Usnea maculata*	100	80
Acetone	*Ramalina conduplicans*	100	100
Acetone	*Rocella montagnei*	100	80
Methanol	*Usnea nilgirica*	100	100
Methanol	*Usnea undulata*	100	100
Methanol	*Usnea maculata*	100	80
Methanol	*Ramalina conduplicans*	100	100
Methanol	*Rocella montagnei*	100	80

Table.3 Effect of *Usnea nilgirica* column fractions on the percentage mortality of the storage pests

Fractions	Percentage of mortality (%)	
	Tribolium castaneum	***Sitophilus oryzae***
1	100	80
2	40	0
3	0	40
4	60	100

Fractions 1 : Ethyl acetate: Methanol 1:3 (12.5:37.5 ml)
Fractions 2 : Ethyl acetate: Methanol 1:1 (25:25 ml)
Fractions 3 : Ethyl acetate: Methanol 3:1 (37.5:12.5 ml)
Fractions 4 : Ethyl acetate: Methanol 1:1 (25:25 ml)

Table.4 Effect of *Usnea undulate* column fractions on the percentage mortality of the storage pests

Fractions	Percentage of mortality (%)	
	Tribolium castaneum	***Sitophilus oryzae***
1	100	0
2	20	80
3	0	0
4	0	20

Fractions 1 : Ethyl acetate: Methanol 1:3 (12.5:37.5 ml)
Fractions 2 : Ethyl acetate: Methanol 1:1 (25:25 ml)
Fractions 3 : Ethyl acetate: Methanol 3:1 (37.5:12.5 ml)
Fractions 4 : Ethyl acetate: Methanol 1:1 (25:25 ml)

Table.5 Effect of *Usnea maculate* column fractions on the percentage mortality of the storage pests

Fractions	Percentage of mortality (%)	
	Tribolium castaneum	***Sitophilus oryzae***
1	100	60
2	100	100
3	100	80
4	100	40

Fractions 1 : Ethyl acetate: Methanol 1:3 (12.5:37.5 ml)
Fractions 2 : Ethyl acetate: Methanol 1:1 (25:25 ml)
Fractions 3 : Ethyl acetate: Methanol 3:1 (37.5:12.5 ml)
Fractions 4 : Ethyl acetate: Methanol 1:1 (25:25 ml)

Table.6 Effect of *Ramalina conduplicans* column fractions on the percentage mortality of the storage pests

Fractions	Percentage of mortality (%)	
	Tribolium castaneum	***Sitophilus oryzae***
1	100	0
2	100	20
3	40	100
4	0	0

Fractions 1 : Ethyl acetate: Methanol 1:3 (12.5:37.5 ml)
Fractions 2 : Ethyl acetate: Methanol 1:1 (25:25 ml)
Fractions 3 : Ethyl acetate: Methanol 3:1 (37.5:12.5 ml)
Fractions 4 : Ethyl acetate: Methanol 1:1 (25:25 ml)

Table.7 Effect of *Rocella montagnei* column fractions on the percentage mortality of the storage pests

Fractions	Percentage of mortality (%)	
	Tribolium castaneum	***Sitophilus oryzae***
1	100	0
2	100	20
3	40	100
4	0	0

Fractions 1 : Ethyl acetate: Methanol 1:3 (12.5:37.5 ml)
Fractions 2 : Ethyl acetate: Methanol 1:1 (25:25 ml)
Fractions 3 : Ethyl acetate: Methanol 3:1 (37.5:12.5 ml)
Fractions 4 : Ethyl acetate: Methanol 1:1 (25:25 ml

Two years later, antifungal properties were observed in extracts of the Andean lichens *Protousnea poeppigii* and *Usnea rigida*, which contain divaricatinic acid, isodivaricatinic acid, usnic acid and 5-resorcinol. Also, Mitrović *et al.,* (2011) determined strong antifungal effect of *Evernia prunastri* and *Hypogymnia physodes*. While *Evernia prunastri* exerted the best effect on yeasts, *Hypogymnia physodes* were better on filamentous fungi. Third, antiviral properties have been attributed to specific lichen secondary metabolites. For instance, Perry *et al.,* (1999) showed antiviral activity of usnic acid against Herpes simplex type 1 and Polio type 1 viruses. Parietin extracted from *Teloschistes chrysophthalmus* proved as virucidal for Junin and Tacaribe arenaviruses. Lichenan, widely distributed in lichens, demonstrated inhibition of tobacco mosaic virus. (Lin *et al*., 2003). Protolichesterinic acid isolated from *Cetraria islandica* showed inhibition of growth of breast cancer cell lines and mitogenstymulated lymphocytes. Inhibition of enzyme 5-lipoxygenase involved in inflammation, non-specific binding to DNA polymerase β and DNA ligase I are possible mechanisms of antitumor activity of protolichesterinic acid. Bucar *et al.,* (2004) revealed inhibitory activities on 12(S)- HETE inside antiproliferative effect of several lichen substances in human platelets. Furthermore, antipyretic and analgesic effects of lichen components were demonstrated on animal studies. For instance, usnic acid from *Usnea diffracta* inhibited acetic-acid-induced writhing in mice and raised the pain threshold in dosedependent manner (Okuyama *et al.,* 1995). Finally, antioxidant properties, already mentioned previously concerned with phenolic content of lichens. Jayaprakasha and Rao (2000) examined antioxidant capacities of methyl orsellinate, atranorin, osellinic acid and lecanoric acid. Bhattarai *et al.,* (2008) noticed stronger antioxidant activities in lichens from Antarctic that the one in lichens from native to temperate or tropical regions. Mitrović *et al.,* (2011) compared the chemical content of lichen extracts (*Flavoparmelia caperata, Evernia prunastri, Hypogymnia physodes* and *Cladonia foliacea*) and their free radical scavenging ability. They observed strong correlation according to previous conclusions of Ranković *et al.,* (2010). *Hypogymnia physodes* with the highest phenolic content showed the strongest antioxidant effect.

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