DISCUSSION

SPORE GERMINATION

Hedwig (1782) published earliest report on spore germination in mosses. Since then many workers studied different aspects of spore germination (Heald, 1898; Correns, 1899; Goebel, 1905; Bauer, 1942; Sironval, 1947; Noguchi, 1958; Nishida, 1973; Nehira, 1976). Most bryologists divide process of spore germination into two phases: (a) Swollen phase (b) Distension phase (Mohr, 1963; Krupa, 1964; Valanne, 1966). In the first phase spore increases in diameter after absorption of water. This is followed by greening of plastids and finally rupturing of exospore. Second phase i.e., distension phase is characterized by emergence of endospore in the form of a germ tube. In the present investigation also in Hydrogonium arcuatum and Anoectangium clarum similar observations were made during spore germination.

A transverse cross wall appears at the base of germ tube in Anoectangium bicolor, Bryum argenteum and Bryum bicolor (Saini, 1994); Bryum capillare and Brachymenium bryoides (Chaturvedi, 2001). However, in some species like Funaria hygrometrica (Allsopp & Mitra, 1958) and Physcomitrium pyriforme (Goebel, 1882; Rabe, 1905; Schoene, 1906; Meyer, 1947; Kachroo, 1954) formation of rhizoidal filaments, in addition to chloronema during spore germination has been reported. No such rhizoidal filament formation occurred in Bryum capillare and Brachymenium bryoides (Chaturvedi, 2001) as well as in Hydrogonium arcuatum and Anoectangium clarum (present investigation).

In many species of mosses such as Anoectangium bicolor (Saini, 1994); A. stracheyanum and Pohlia elongata (Vashistha, 1986) spores show dormancy and do not germinate in the same season. In these species variations in light intensity and degree of hydration were ineffective in inducing spore germination. Germination was induced by a cold - treatment of spores or by exogenous application of kinetin (Vashistha & Chopra, 1987). Spores do not show any dormancy and readily germinate on NB medium under ordinary cultural conditions in Bryum capillare and Brachymenium bryoides (Chaturvedi, 2001) as well as in Hydrogonium arcuatum and Anoectangium clarum (present investigation).
Takao (1976) reported that in *Bartramia pomiformis*, spore germination was promoted under low light intensity when sucrose was added to the medium. However, addition of sucrose and variation in its concentration was ineffective in inducing spore germination in *Anoectangium bicolor* (Saini, 1994). In present investigation, spore germination was promoted in *Hydrogonium arcuatum* when sucrose was added to the medium but was inhibited in *Anoectangium clarum*.

**PROTONEMAL DIFFERENTIATION**

Many bryologists observed existence of two principal types of filaments of moss protonema differing in morphological characteristics and direction of growth. (Müller 1874; Sachs, 1875; Goebel, 1882; Correns, 1899). Sironval (1947) studied protonemal development in the moss *Funaria hygrometrica* and reported two distinct development stages: chloronema and caulonema. The first stage chloronema is produced on germination of spores and is characterized by filaments having hyaline cell walls, perpendicular cross walls, irregular branching and numerous round chloroplasts. The second stage or the caulonema is characterized by filaments having brown cell wall, oblique cross walls and a few unevenly distributed elongated chloroplasts. These two developmental stages of protonema have been observed in majority of the investigated mosses (Van Andel, 1952; Bopp, 1954, 1961; Kofler, 1956; Allsopp & Mitra, 1958; Vashistha & Chopra, 1987; Sarla, 1987; Dhingra-Babbar, 1990; Saini, 1994). In the present investigation also protonema of both species show distinct chloronemal and caulonemal stages. However, in *Leucodon sciuroides* (Lal, 1961) and *Physcomitrium turbinatum* (Nebel & Neylor, 1968a) no stage comparable to caulonema was reported.

Sironval (1947) opined that caulonema arises from persisting apical cells of degenerating chloronema. Subsequent bryologists did not accept his views regarding development of caulonema from chloronema. Development of caulonema from chloronema was regarded a continuous process by these bryologists (Van Andel, 1952; Bopp, 1954, 1955, 1961; Kofler, 1956; Allsopp & Mitra, 1956, 1958; Saini, 1994; Chaturvedi, 2001; Vashistha & Chopra, 1987). In present investigation on *Hydrogonium arcuatum* and *Anoectangium clarum* protonema exhibited these two developmental stages i.e. chloronema and caulonema. In both the species a gradual
transformation of chloronema into caulonema has been observed. However, in some species like *Leucodon sciuroides* (Lal, 1961), *Pogonatum aloides* (Sood, 1975) and *Pylaisiella selwyni* (Spiess et al., 1984) only chloronema stage has been reported. In contrast, in *Physcomitrium turbinatum* (Nebel & Naylor, 1968a) chloronema-like stage was not observed. On the basis of her studies on cultured protonema of *Funaria hygrometrica* under variable cultural conditions Kofler (1956) concluded that the appearance and extent of caulonema formation depends on cultural conditions as well as composition of the medium. In many species like *Funaria hygrometrica* and *Ceratodon purpureus* caulonema formation fails to occur under certain conditions such as low light intensity (Bopp, 1959a; Lehnert, 1982), low temperature (Bopp, 1959a), liquid medium (Szweykowska, 1962; Johri, 1974) or low concentration of agar (Kofler, 1959) or partly hydrolysed agar (Hatanaka-Ernst, 1966). Caulonema formation was favoured at higher temperature in *Funaria hygrometrica* and *Weissia controversa* (Dietert, 1980) and *Anoectangium bicolor*, *Bryum argenteum* and *Bryum bicolor* (Saini, 1994), *Bryum capillare* and *Brachymenium bryoides* (Chaturvedi, 2001).

Kanda and Nehira (1976) reported that the protonema of most of the pleurocarpous mosses do not exhibit heterotrichous habit which is more common in the protonema of acrocarpous species.

**BUD FORMATION**

Many bryologists suggested that formation of caulonema is a pre-requisite for initiation of buds on the protonema on *Funaria hygrometrica* (Sironval, 1947; Bopp, 1959 a,b; Brandes & Kende, 1968). Similar observations have been made in *Bryum klinggraeffii* (Rawat & Chopra, 1976), *Timmiella anomala* (Chopra & Rekhi, 1979), *Anisotrichium spirale* and *Pohlia elongata* (Vashistha & Chopra, 1987). In the present investigation also, buds were initiated on caulonemal filaments of the investigated species. In contrast, Kofler (1963) opined that caulonema is not obligatory for bud formation. Buds are formed on filaments with chloronemal characteristics in *Leucodon sciuroides* (Lal, 1961) and *Microdus miquelianus* (Nair & Raghavan, 1976).

In *Entodon myurus* and *Polytrichum juniperinum* (Nehlsen, 1979) buds were produced directly on germinating spores in the presence of cytokinin in the medium (Sood & Chopra, 1973). Yoshida and Yamamoto (1982) after studying the process of bud formation in *Physcomitrium sphaericum* opined that bud formation is influenced by the position of cells from spore. They observed that in this species even caulonema cells which are at positions less than 15 cells away from a spore never formed buds, whereas chloronema cells present near the base of a lateral filament formed buds.

In some mosses like *Funaria hygrometrica* (Bopp & Brandes, 1964) and *Timmiella anomala* (Chopra & Rekhi, 1979) protonema produced buds after attaining a critical size. In contrast, there is no correlation between protonemal growth and bud initiation in *Physcomitrium turbinatum*. (Nebel & Naylor, 1968 a, b), *Anoectangium stracheyanum*, *Anisotrichium spirale*, *Pohlia elongata* (Vashistha, 1986), *Anoectangium bicolor*, *Bryum bicolor*, *Bryum argenteum* (Saini, 1994), *Bryum capillare* and *Brachymenium bryoides* (Chaturvedi, 2001). In the present investigations on protonemal growth and bud formation in *Hydrogonium arcuatum* and *Anoectangium clarum* no correlation was observed between size of protonemal patch and bud initiation.
Age of protonema rather than its size has been reported to be a critical factor for bud initiation in *Pylaisiella selwyni* (Spiess et al., 1971) and *Anisothecium molliculum* (Kumra & Chopra, 1985) and *Entodon laetus* (Awasthi et al., 2010). Formation of buds on germinating spores in *Entodon myurus* (Sood & Chopra, 1973) and *Polytrichum juniperinum* (Nehlsen, 1979) and also on five-celled long protonemal filaments of *Funaria hygrometrica* on cytokinin-supplemented media, suggest that bud formation is neither dependent on critical area of protonemal patch nor on its age, but it is dependent on the physiological maturity of individual cells/filaments (Bopp & Dickmann, 1967).

**PHYSICAL FACTORS**

**Light**

The significance of light in bud formation was realized by Klebs (1893) in *Funaria hygrometrica*. He observed that buds are not produced on protonema in dark. Subsequent bryologists (Goebel, 1896; Servettaz, 1913; Ubisch, 1913) made similar observations. Pringsheim and Pringsheim, (1935) also reported that in *Funaria hygrometrica* addition of carbon and nitrogen failed to induce buds in dark. However, a few workers reported formation of buds on protonema growing in dark (Fries, 1945; Keil, 1949; Belkengren, 1962), but these reports were criticized by subsequent workers due to defective methodology. Naef and Simon, (1980) reported that in *F. hygrometrica* bud formation can occur in dark if cultures are exposed to light for some time before transferring cultures in dark. Buds failed to appear in dark even in the presence of sucrose in *Bryum capillare* and *Brachymenium bryoides* (Chaturvedi, 2001) and *Anoectangium stracheyanum* (Vashistha, 1986) as well as in the present investigation on *Hydrogonium arcuatum* and *Anoectangium clarum*. Bopp and Brandes (1964) observed that in *Funaria hygrometrica* sugar promoted bud initiation only under suboptimal conditions of light. Buds were produced only in light in *Anoectangium bicolor, Bryum bicolor* and *Bryum argenteum* (Saini, 1994).

Studies on the effect of light intensity on the process of bud formation in *Funaria hygrometrica* suggested that for bud initiation on the protonema relatively higher light intensity is required (Bopp, 1959a; Kofler et al., 1963; Lehnert, 1982). In *Barbula gregaria* (Kumra, 1981). *Anisothecium spirale, Pohlia elongata* (Vashistha
& Chopra, 1987), Microdus brasiliensis (Mehta, 1988), Timmiella anomala (Kapur, 1990), Bryum capillare and Brachymenium bryoides (Chaturvedi, 2001) buds were initiated only in light and their number increased with increase in light intensity up to 3,500 lux. Variation in light intensity was ineffective in inducing buds in Anoectangium stracheyanum (Vashistha, 1986), Racomitrium lanuginosum, Dicranella coarctata (Kumra, 1981) and Anoectangium bicolor (Saini, 1994).

Increase in light intensity had no appreciable effect on bud formation in Brachymenium bryoides (Chaturvedi, 2001). In the present investigation also, increase in light intensity had no appreciable effect on bud formation in Anoectangium clarum. Mitra and Allsopp (1959a) reported that light plays an important role in the synthesis of a formative factor which is required for initiation of buds on moss protonema. Nebel and Naylor (1968a) observed that bud formation in Physcomitrella patens is dependent upon a threshold level of some morphogenetic substance for which a cumulative light dose is necessary. Larpent-Gourgaud, (1969) opined that light plays an important role in the synthesis of a “ramification factor” which is responsible for ramification of protonema and bud initiation. According to Chopra and Gupta, (1967) in Funaria hygrometrica light plays non-photosynthetic as well as photosynthetic roles. The non-photosynthetic role of light is responsible for initiation of buds and photosynthetic role is responsible for growth of primordia. In Anisothecium spirale, Pohlia elongata (Vashistha, 1986), Bryum argenteum (Saini, 1994) and Bryum capillare (Chaturvedi, 2001) thin gametophores with reduced leaves are produced under low intensity of light, whereas comparatively thicker shoots with larger leaves developed under higher light intensity. Similar observations have been made in present investigation on Hydrogonium arcuatum.

**Hydration**

Effect of hydration on shoot morphology in mosses was reported for the first time by Bastit (1891). He observed that in Polytrichum juniperinum degree of hydration influenced the height, direction of growth and also arrangement, size and colour of leaves. Servettaz (1913) observed differences in the morphology of aerial and aquatic forms of the moss Hypnum velutinum. Gurlitt (1918) and Brown (1919) noticed that in liquid medium buds were formed only near the wall of culture vessels.
According to these workers in this position protonemal filaments are able to get support to maintain themselves above the surface of liquid medium. Meyer (1940) observed that in *Funaria hygrometrica* bud number is more on solid medium in comparison to liquid medium. According to Kumra (1981) different species require varying degree of hydration for optimum bud formation. Species like *Barbula gregaria* (Kumra, 1981) and *Timmiella anomalala* (Kapur, 1983) produced maximum number of buds in liquid cultures and on semi solid media number of buds decreased with increase in concentration of agar in the medium. However, *Bryum coronatum* produced maximum buds at 1.6% agar (Kumra, 1981). For most of the investigated species 0.8% agar proved optimum for bud number: *Anisothecium molliculum* (Kumra & Chopra, 1985), *Garckeia phasocoides* (Sarla, 1987), *Pohlia elongata*, *Anisothecium spirale* (Vashistha & Chopra, 1987), *Microdus brasiliensis* (Mehta, 1988), *Bryum capillare* and *Brachymenium bryoides* (Chaturvedi, 2001). In the present investigation also, in *Hydrogonium arcuatum* maximum number of buds were produced on media containing 0.8% agar and further increase in concentration of agar resulted in reduction in bud number. According to Slabecka-Szwtkowska (1955), in general, plants exhibit normal growth and development on media containing 0.8% to 1.6% agar, and at higher concentrations of agar diffusion of carbohydrates become difficult.

**CHEMICAL FACTORS**

**Sugars**

Sugars are known to support growth of protonema in dark (Servettaz, 1913; Ubisch, 1913). Robbins (1918) observed that addition of sugar to the medium was able to enhance growth of protonema but was not effective in induction of buds. Keil (1949) reported that in the moss *Splachnum* sp. addition of sucrose in the medium resulted in the formation of buds on the protonema even in dark but his report was doubted by subsequent workers due to defective methodology. Pringsheim and Pringsheim (1935) observed that in *Funaria hygrometrica* addition of 4.0% sucrose caused inhibition of bud formation. In *Timmiella anomalala* addition of sucrose failed to substitute non-photosynthetic effect of light on bud initiation (Kapur, 1983). In *Pohlia nutans* number of buds increased with increase in concentration of sucrose up
to 2% and further increase in sucrose caused reduction in bud number (Mitra & Allsopp, 1959a). Sucrose at 1.0% has been reported to induce maximum number of buds in *Anisothecium molliculum* (Kumra & Chopra, 1985), *Garckea phascoides* (Sarla, 1987), *Microdus brasiliensis* (Mehta & Chopra, 1991), *Anisothecium spirale, Pohlia elongata* (Vashistha & Chopra, 1987), *Barbula horricomis* (Saini, 1994), *Bryum capillare, Brachymenium bryoides* (Chaturvedi, 2001) and *Funaria hygrometrica* (Awasthi et al., 2009) and at higher concentration number of buds decreased. In the present investigation also in *Hydrogonium arcuatum* maximum buds were produced on media containing 1.0% sucrose and at higher concentration number of buds decreased. Variation in sucrose level was ineffective in inducing buds in *Anoectangium stracheyanum* (Vashistha, 1986), *Anoectangium bicolor* (Saini, 1994) as well as in *Anoectangium clarum* (present investigation).

It has been suggested that presence of sugar in the medium helps in the attainment of a certain minimal sugar concentration in the protonema which may be pre-requisite for initiation of buds (Mitra & Allsopp, 1959b, c). On the basis of observations on *Funaria hygrometrica* that under optimal condition of light, addition of sugar to the medium has little or no effect on the process of bud formation. Bopp and Brandes (1964) suggested that in this species sugars help in attaining the critical size necessary for bud initiation. In *Physcomitrium coorgense* sucrose is probably involved in the determination of an apical cell with two cutting faces (sporophytic) or three cutting faces (gametophytic) (Menon & Lal, 1974). In *Bryum argenteum* medium containing 1.5% sucrose elicited maximum response (Sabovljevic et al., 2005).

**Auxins**

Studies of Hatanaka-Ernst (1966) on mutants of *Funaria hygrometrica* clearly demonstrated importance of auxin in the process of bud formation. She raised certain mutants which formed buds only when the medium was supplemented with auxin. Exogenous application of auxins in mosses has been shown to elicit variable effects in different species. In many species such as *Ceratodon purpureus* (Szweykowska, 1962), *Anoectangium thomsonii* (Chopra & Rashid, 1969a), *Dicranella coarctata* (Kumra, 1981), *Bryum atrovirens* (Chopra & Vashistha, 1990) and
*Anoectangium bicolor* (Saini, 1994) buds are not produced under ordinary cultural conditions and exogenous application of auxin induced buds. Similar observations has been made in *Anoectangium clarum* in the present investigation. In some other species in which bud formation occurs spontaneously under ordinary cultural conditions, addition of auxin to culture medium has been shown to enhance number of buds. This type of effect has been reported in *Funaria hygrometrica* (Hurel-Py, 1948; Prusińska *et al.*, 1969; Sood & Hackenberg, 1979), *Timmiella anomala* (Kapur, 1983), *Anisothecium molliculum* (Kumra & Chopra, 1985), *Gararia phascoides* (Chopra & Sarla, 1986), *Pohlia elongata* (Vashistha, 1986), *Bryum capillare* and *Brachymenium bryoides* (Chaturvedi, 2001). In the present investigation in *Hydrogonium arcuatum* addition of auxins resulted in enhancement of bud number. In some species application of auxins was not effective in induction of buds on the protonema. This category includes *Hyophila involuta* (Rahbar & Chopra, 1982), *Pogonatum aloides*, (Sood, 1975) and *Racomitrium lanuginosum* (Kumra, 1981).

Not all auxins are equally effective as bud inducers. Their effectiveness varies in different species. In *Barbula gregaria*, NOA was most effective and it was followed by 2,4-D, NAA, IAA, and IBA. In *Dicranella coarctata* IAA, NOA, NAA and 2,4-D were effective in decreasing order (Kumra, 1981). In *Anisothecium molliculum*, of the four auxins tested (IAA, NAA, NOA and 2,4-D), NAA proved most effective (Dua, 1983). In *Timmiella anomala* bud formation was stimulated only by IAA and NAA whereas 2,4-D and NOA proved inhibitory (Kapur, 1983). Vashistha (1986) observed that in *Anisothecium spirale*, NOA, 2,4-D, NAA and IAA were effective in decreasing order. However, in *Pohlia elongata* only 2,4-D and NOA stimulated bud formation. In another moss, *Anoectangium stracheyanum*, of the four auxins (IAA, NAA, NOA & 2,4-D) tested only 2,4-D was able to induce normal buds, whereas NOA induced unstable buds. However, in *Anoectangium bicolor* only IAA induced normal stable buds and NAA resulted in formation of unstable buds (Saini, 1994). In *Bryum capillare* 2,4-D proved most effective in bud formation and it was followed by IAA, NOA and NAA in decreasing order and in *Brachymenium bryoides* also 2,4-D elicited maximum response and was followed by NOA (Chaturvedi, 2001).
In the present investigation, in *Hydrogonium arcuatum* 2,4-D proved most effective in bud formation and it was followed by IAA, NOA and NAA in decreasing order. In *Anoectangium clarum* also 2,4-D elicited maximum response.

Kofler (1951) reported that in *Funaria hygrometrica* buds developed at high concentrations of 2, 4-D remained undifferentiated. Similarly in *Anisothecium* buds formed at higher concentrations of auxins either failed to develop further or formed stunted gametophores. In *Anoectangium bicolor* only IAA induced normal stable buds, whereas unstable buds were induced by NAA. Of the four auxins (IAA, NAA, NOA & 2,4-D), IAA proved most effective in *Bryum bicolor* as well as in *Bryum argenteum* (Saini, 1994).

Auxins also influence morphology of gametophores and at their higher concentrations cause abnormal development. Kofler (1959) observed that in *Funaria hygrometrica* abnormal leafless gametophores with abundant rhizoids are produced at higher concentrations of 2,4-D. Szweykowska (1962) reported that higher concentrations of auxin disturbed the normal development of gametophores in the moss *Ceratodon purpureus*. Kumra (1981) also observed formation of abnormal shoots in *Dicranella coarctata* on medium supplemented with higher concentrations of auxin. Production of stunted gametophores with abundant rhizoid-like filaments has been reported in species like *Anisothecium spirale*, *Pohlia elongata* (Vashistha, 1986), *Bryum atrovirens* (Chopra & Vashistha, 1990) and *Anoectangium bicolor* (Saini, 1994). In *Bryum capillare* and *Brachymenium bryoides* production of stunted shoots with abundant rhizoids has been observed at higher concentrations of auxins in the medium. In the present investigation also production of stunted shoots with abundant rhizoids has been observed in *Hydrogonium arcuatum* as well as in *Anoectangium clarum* at higher concentrations of auxins in the medium.

Bopp (1953) reported dedifferentiation of buds in *Funaria hygrometrica* at higher concentrations of auxins. Similar observations were made in *Tortella caespitosa* (Gorton & Eakin, 1957). Dedifferentiation of buds at higher concentration of NAA in the medium has been reported in *Anoectangium stracheyanum* (Vashistha, 1986) and *A. bicolor* (Saini, 1994).
Using auxin-deficient mutants of *Funaria hygrometrica* attempts have been made to understand the role of endogenous auxin in bud formation (Hatanaka-Ernst, 1966; Atzorn et al., 1989 a, b; Bhatla & Bopp, 1992; Kapoor & Bhatla, 1993). According to (Bopp, 1982; Lehnert & Bopp, 1983) exogenously applied auxins and auxin precursors (tryptophan, indolepyruvic acid & indoleacetaldehyde) are responsible for formation of caulonema which act as target cell for initiation of buds. Exogenous application of auxins has been shown to elicit varying effects in *Funaria hygrometrica* (Hurel-Py, 1948), *Tortella caespitosa* (Gorton & Eakin, 1957), *Barbula gregaria*, *Bryum coronatum* (Kumra, 1981), *Anisothecium spirale*, *Pohlia elongata* (Vashistha, 1986), *Garckeia phascoides* (Sarla, 1986), *Anisothecium molliculum* (Dua 1983) and also in *Barbula horricomis* (Saini, 1994). Auxins at lower levels enhanced bud formation and higher levels inhibited this response. Ashton and co workers reported that in *Physcomitrella patens* a low concentration of auxins is required for formation of caulonema and further growth of protonema (Ashton et al., 1979a, b; Cove & Ashton, 1984). Auxin at higher concentration interacts with cytokinin and affects further development in two ways: (i) auxin and cytokinin are required together to induce caulonemal side branch initials to form buds and (ii) these two hormones (auxin & cytokinin) also act together to suppress the proliferation of secondary chloronema filaments. It has been suggested that in *F. hygrometrica* auxin cause caulonema differentiation by modulating changes in the intracellular free (Ca$^{2+}$) and calmodulin activation (Bhatla et al., 1998). Indirect evidence for an auxin-receptor in moss protonema was provided by (Bhatla & Bopp, 1992) and Johri & Panigrahi (1998) characterized an auxin-binding protein from the protonema of *Funaria hygrometrica*. In *Pogonatum urnigerum*, auxin IAA favoured bud formation and the cytokinin 6-BA restrained gametophore growth (Cvetic et al., 2007).

**Cytokinins**

Responses of mosses to cytokinins can be grouped into three categories. In first type, application of cytokinin induces buds in those species which fail to produce buds under ordinary cultural conditions. Such species are: *Anoectangium thomsonii* (Chopra & Rashid, 1969a), *Microdus miquelianus* (Nair & Raghavan, 1976), *Pogonatum aloides* (Sood, 1972), *Anoectangium stracheyanum* (Vashistha, 1986) and
A. bicolor (Saini, 1994). In the second type of response, exogenously applied cytokinins cause increase in number of buds as well as reduction in time taken for bud initiation in species in which bud formation occurs spontaneously. This type of response has been observed in Splachnum ampullaceum (Maltzahn, 1959), Funaria hygrometrica (Bopp, 1963; Iwasa, 1965), Entodon myurus (Sood & Chopra, 1973), Pylaisiella selwynii (Spiess et al., 1973), Polytrichum juniperinum (Nehlsen, 1979), Timmiella anomala (Chopra & Rekhi, 1979), Garckea phascoides (Chopra & Sarla, 1986), Philonotis lancifolia (Chopra & Dhingra-Babbar, 1988), Bryum bicolor, Bryum argenteum (Saini, 1994), Bryum capillare and Brachymenium bryoides (Chaturvedi, 2001). In the present investigation also, applied cytokinins advanced bud formation and considerably enhanced number of buds in Hydrogonium arcuatum. In some species like Tortella caespitosa (Gorton & Eakin, 1957) and Amblystegium riparium (Kato et al., 1980) although kinetin increased bud number but did not affect the time of bud initiation. In the third category are included species in which cytokinins induce buds under conditions unfavourable for bud initiation such as low light intensity, blue light or dark. In Pohlia nutans, cytokinin induced buds in low light intensity and in blue light (Mitra et al., 1959c; 1962; 1965). Similarly, in Funaria hygrometrica kinetin induced buds under blue light which is inhibitory for bud formation (Jahn, 1964). Szweykowska (1963) reported that in Ceratodon purpureus kinetin induced buds even in dark. In Funaria hygrometrica Chopra and Gupta (1967) observed bud formation on medium lacking sucrose, in dark, in the presence of kinetin. In most of the cases, cytokinins were able to induce buds after some protonemal growth has occurred (Spies, 1976; Vijelovic & Sabovljic, 2003). However, in Entodon myurus (Sood & Chopra, 1973) and Polytrichum juniperinum (Nehlsen, 1979) buds appeared shortly after spore germination on a cytokinin supplemented medium. In response to cytokinins formation of buds on a five-celled long caulonemal filaments of Funaria hygrometrica has also been reported (Bopp & Dieckmann, 1967).

Although cytokinins greatly increase number of buds produced on moss protonema, but their higher concentration, adversely affect the morphology of buds. Gorton and Eakin (1957) observed formation of abnormal buds in Tortella caespitosa at higher concentrations of cytokinins. Such abnormal structures produced on
cytokinin-supplemented media are known as moruloid buds or cabbage heads or callus-like masses etc. Production of moruloid buds, in response to higher concentrations of cytokinins has been observed in Funaria hygrometrica (Szweykowska & Maćkowiak, 1962), Anoectangium thomsonii (Chopra & Rashid, 1969a), Bryum klinggraeffii (Rawat & Chopra, 1979), Amblystegium riparium (Kato et al., 1980), Pylaisiella selwynii (Spiess, 1976), Anisothecium spirale (Vashistha, 1986), Philonotis lancifolia (Chopra & Dhingra Babbar, 1988), Anoectangium bicolor, Bryum bicolor, Bryum argenteum (Saini, 1994), Bryum capillare and Brachynemium bryoides (Chaturvedi, 2001). In the present investigation also in Hydrogonium arcuatum and Anoectangium clarum all the tested cytokinins induced moruloid buds in higher concentrations.

Effectiveness of different cytokinins in bud formation varies in different species. Rashid (1968) reported that in Anoectangium thomsonii cytokinin SD-8339 was most effective and it was followed by 2iP, Kinetin and BAP. In Funaria hygrometrica, 2iP was most effective in bud formation and it was followed by Kinetin, and BAP in decreasing order (Szweykowska et al., 1969; 1971; 1972). In Entodon myurus 2iP was most effective and tricanthine the least (Sood, 1972). In Polytrichum juniperinum also, of the three cytokinins (2iP, Kinetin & Zeatin), 2iP proved most effective (Nehlsen, 1979). BAP elicited maximum response in Bryum pallescens (Sarla & Chopra, 1985), Anoectangium stracheyanum (Vashistha, 1986). Bryum argenteum, B. bicolor and Anoectangium bicolor (Saini, 1994). In Pohlia elongata kinetin induced maximum buds (Vashistha, 1986) and in Bryum capillare and Brachynemium bryoides, 2iP proved most potent bud inducer (Chaturvedi, 2001). This may be due to its endogenous occurrence in mosses. In the present investigation in Hydrogonium arcuatum and Anoectangium clarum, 2iP proved most potent bud inducer. Presence of 2iP has been demonstrated in the callus of hybrid of Funaria hygrometrica X Physcomitrium pyriforme (Beutelmann & Bauer, 1977) and Physcomitrella patens (Wang et al., 1980; 1981a, b). The catabolism of the different cytokinins involves different pathways in moss protonema. It has been reported that Kinetin and BAP are metabolized very quickly to adenine and adenine derivatives by a direct cleavage of the side chain, whereas 2iP is more stable (Erichsen et al., 1978; Bopp & Erichsen, 1981; Bopp & Gerhätiser, 1985; Reutter et al., 1998).
Initiation of buds on the moss protonema is comparable to formation of shoot-buds in cultures of higher plants of cytokinin supplemented medium as demonstrated by Miller and Skoog (1953) in tobacco callus. As in higher plants, in mosses also cytokinins delay senescence of protonema. Protonemata of *Bryum capillare* and *Brachymenium bryoides* remained green for longer duration on cytokinin-supplemented media (Chaturvedi, 2001). In the present investigation too, protonemata of *Hydrogonium arcuatum* and *Anoectangi um clarum* remained green for longer duration on cytokinin-supplemented media. According to (Richmond & Lang, 1957) kinetin stabilizes chlorophyll content and chloroplast structure.

Analysis of mutants of *Physcomitrella patens* revealed that both endogenous auxin and cytokinin play interdependent roles in several steps of gametophytic development (Ashton *et al.*, 1979 a, b; Cove & Ashton, 1984). Induction of buds only by the combination of auxin and cytokinin in *Pogonatum aloides* (Sood, 1975) and *Dicranella coarctata* (Kumra, 1981) supported the view of Ashton and co-workers. Bopp (1982) suggested that auxin creates target cells and maintain their differentiation, whereas cytokinin acts on these cells to induce buds. Lehnert and Bopp (1983) reported that the interaction of two hormones is a sequential interaction and cytokinin can act only if auxin has already done its work.

Changes in cytosolic \( \text{Ca}^{2+} \) in the response to cytokinin treatment, have been reported by some workers (Saunders & Hepler, 1981; 1982; 1983; Saunders, 1986; Conrad & Hepler, 1988; Conrad *et al.*, 1986). Bud number is found to be regulated by the concentration of exogenous cytokinin as incipient buds or bud initials become stably committed buds (Christianson, 1998; Christianson & Hornbuckle, 1999).

**Gibberellins**

Gibberellins have been shown to elicit variable effects in different species. In some species exogenous application of gibberellic acid stimulated bud formation. This type of response has been observed in *Splachnum ampullaceum* (Maltzahn & Mac Quarrie, 1958), *Pohlia nutans* (Mitra & Allsopp, 1959 b,c), *Anoectangium thomsonii* (Rashid, 1970), *Barbula gregaria* (Kumra, 1981), *Timmiella anomala* (Kapur, 1983), *Garckeia phascoide* (Sarla, 1987) , *Microdus brasiliensis* (Chopra & Mehta, 1992), *Bryum bicolor* and *Bryum argenteum* (Saini, 1994). \( \text{GA}_3 \) enhanced bud
number in *Bryum capillare* and *Brachymenium bryoides* (Chaturvedi, 2001) specially at lower levels. In the present investigation also GA$_3$ enhanced bud number in *Hydrogonium arcuatum* and *Anoectangium clarum*, especially at lower levels.

Jahn (1964) reported that in *Funaria hygrometrica*, GA$_3$ increased bud number but delayed their initiation. In *Pohlia nutans* (Mitra & Allsopp, 1959 b,c), *Anisotheicum molliculum* (Dua, 1983), *Pohlia elongata* (Vashistha, 1986) gibberellic acid advanced bud formation. In *Bryum capillare* and *Brachymenium bryoides* bud formation was advanced only at lower levels (Chaturvedi, 2001). GA$_3$ has been shown to induce buds in species which do not form buds under ordinary cultural conditions: *Anoectangium thomsonii* (Rashid, 1970) and *Anoectangium stracheyanum* (Vashistha, 1986). In *Entodon myurus* gibberellic acid reduced the time taken for bud initiation without appreciably affecting the bud number.

Gibberellic acid was ineffective in bud induction in *Amblystegium riparium* (Belkengren, 1962), *Microdus miquelianus* (Nair & Raghavan, 1976), *Ceratodon purpureus* (Szweykowska et al., 1972), *Campylopus richardii* (Chopra & Mehta, 1991) and *Anoectangium bicolor* (Saini, 1994).

Muromtsev et al., (1964) reported presence of some gibberellin-like substances in mosses. But according to Bopp (1990), to date there have been no reports demonstrating the occurrence of gibberellins in mosses, especially in the protonema of *Funaria hygrometrica*. In this species he failed to detect gibberellins with different bioassays.

In *Bryum capillare* and *Brachymenium bryoides*, GA$_3$ stimulated bud formation only at 10$^{-8}$ M and proved inhibitory at highest concentrations (Chaturvedi, 2001). In the present investigation, GA$_3$ stimulated bud formation only at 10$^{-8}$ M and proved inhibitory at highest concentrations. GA$_3$ delayed bud formation in *Bryum coronatum* (Kumra, 1981) as well as in *Barbula horricomis* (Saini, 1994) especially at higher levels. In *Anisotheicum molliculum* this hormone had no effect on protonemal growth but it reduced the time required for bud formation by 5 days (Dua, 1983). Bud formation was advanced only at lower levels in *Bryum bicolor* and *Bryum argenteum* (Saini, 1994).
Heavy Metals

Bryophytes have little or no ability to avoid uptake and retention of heavy metal particles or ions from the atmosphere, water and soil due to their great ion exchange capacity, absence of cuticle in the gametophyte and simple organization of tissue. Due to these reasons they are extensively used as bioindicators of environmental pollution (Le Blanc, 1961; Rao & Le Blanc, 1967; Gilbert, 1968; Grodzinska, 1978; Rao, 1982; Brown, 1984; Tyler, 1990; Tuba & Csintalan, 1993; Uhlirova et al., 1995; Brog et al., 1996; Brown & Brumelis, 1996; Pott & Turpin, 1996; Bruns et al., 1997; Gerdl et al., 2000). Czarnowska and Rejment-Grochowska (1974) reported that gametophores of mosses in natural habitat contain heavy metals many time higher than those in higher plants. Very little is known about the mechanism of pollution damage (Pott & Turpin, 1996; Gerdl et al., 2000). The specific nature and interaction of the heavy metal with plant shoots, influences their cellular distribution (Well & Brown 1987). In Hylocomium splendens the efficiency order is Cu, Pb>Ni>Co>Zn, Mn (Rühling & Tyler, 1970) and valid for a wide range of concentrations.

Lead- According to Chisholm (1971) lead is one of the most toxic metal and a protoplasmic poison. However, many species of bryophytes have been shown to be tolerant to very high concentrations of lead which are toxic to other groups of plants. Bryophytes which are known to accumulate higher levels of lead include Marchantia polymorpha (Briggs, 1972), Grimmia donniana (Brown & Bates, 1972), Ceratodon purpureus, Eurhynchium praelongum, Plagiommium cuspidatum, Calligeron cuspidatum (Lee, 1972), Rhytidiadelphus squarrosus (Skaar et al.,1973; Gullóag et al.,1974), Hylocomium splendens (Rühling & Tyler, 1970), Sphagnum sp.(Pakarinen & Tolonen, 1976), Leucobryum glaucum (Groet, 1976), Pleurozium schreberi (Barclay-Estrup & Rinnie, 1978), Hypnum cupressiforme (Thomas, 1979), Fontinalis antipyretica (Siebert et al.,1996), Polytrichum juniperinum (Kakulu, 1993), Isothecium stoloniformis (Pott & Turpin, 1998) and Hygrohypnum ochraceum. In leaf cells of Rhytidiadelphus squarrosus lead has been shown to enter into the cytoplasm by pinocytosis and it binds with nuclear membrane (Gullóag et al., 1974). Skaar et al. (1973) suggested that binding of lead as a non-diffusible complex probably reduces
the concentration of diffusible lead in the cytoplasm and thus protect mitochondria and other lead sensitive systems of the cytoplasm from its toxic effects.

In contrast, many species are sensitive to higher concentrations of lead. In such species growth and development are adversely affected. Coombes and Lepp (1974) reported that in *Funaria hygrometrica* protonemal growth was inhibited at higher levels of lead, whereas in the liverwort *Marchantia polymorpha* lead retarded development of gemmalings. Krupsinska (1976) also observed severe disturbance of branching pattern and degeneration of chloroplasts in *Funaria hygrometrica*. In *Semibarbula orientalis* lead has inhibitory effect on protonemal growth and bud formation. In this species the order of toxicity was lead nitrate > lead acetate > lead nitrate + lead acetate (Sharma & Chopra, 1987). In *Timmiella anomala* (Kapur & Chopra, 1989), and *Anoectangium bicolor* and *Bryum argenteum* (Saini, 1994) lead adversely affected protonemal growth and bud formation. At higher concentrations it caused formation of spherical cells. In *Bryum capillare* and *Brachymenium bryoides* lead at all levels caused inhibition of protonemal growth and bud initiation and at higher concentrations protonema showed formation of brood cell-like structures (Chaturvedi, 2001). In the present investigation in *Hydrogonium arcuatum* and *Anoectangium clarum* lead at all levels caused inhibition of protonemal growth and bud initiation and at higher concentrations protonema showed formation of brood cell-like structures. Krzeslowska et al. (1994) and Basile et al. (1995) also reported formation of aberrant forms in *Funaria hygrometrica* due to adverse effects of lead on protonemal shape and size.

**Cadmium-** Many workers reported accumulation of cadmium in large quantities by *Sphagnum* spp. (Pakarinen & Tolonen, 1976; Simola 1977 b,c; Glooschenko, 1989). Other bryophytes which have been known to accumulate cadmium include *Dicranoweissia cirrata* (Johnsen et al., 1983), *Rhytidiadelphus squarrosus* (Brown & Beckett, 1985), *Hylocomium splendens*, *Pleurozium schreberi* (Brog & Steinnes, 1997), *Hygrohypnum ochraceum*, (Carter & Porter, 1997) and *Fontinalis antipyretica* (Bruns et al.,1997), *Isothecium stoloniferum* (Pott & Turpin, 1998) and *Hypnum cupressiforme* (Thomas, 1986). On the other hand, some species are sensitive to cadmium and in these species various stages of growth and
development are inhibited to varying degrees. In *Funaria hygrometrica* cadmium at higher concentrations inhibited spore germination (Lepp & Roberts, 1977). In *Pseudoscleropodium purum* also it had inhibitory effects on growth and development (Haseloff, 1979; Haseloff & Winkler, 1980). In *Polytrichum commune* for spore germination and protonemal growth cadmium proved more toxic than zinc (Francis & Peterson, 1989). Inhibitory effect on protonemal growth and bud formation has also been observed in *Anoectangium bicolor* (Saini, 1994), *Timmiella anomala* (Kapur & Chopra, 1989) and *Barbula horricomis* (Saini, 1994).

Miller and Skoog (1953) reported that very low levels of cadmium either enhances the cofactor function of zinc or release zinc from non-functional binding sites. Jones *et al.* (1975) failed to observe any relationship between cadmium and zinc.

In general protonemata of mosses exhibited various abnormalities like reduction in cell length and change in shape, irregular branching etc. These cells tend to assume spherical shape and are morphologically similar to brood cells as in *Anoectangium bicolor*, *Bryum argenteum* (Saini, 1994), *Bryum capillare*, *Brachymenium bryoides* (Chaturvedi, 2001), *Funaria hygrometrica* (Coombes & Lepp, 1974; Basile *et al.*,1995), *Timmiella anomala* (Kapur & Chopra, 1989) and *Hydrogonium arcuatum* and *Anoectangium clarum* (present investigation). Coombes and Lepp (1974) suggested that formation of such brood cells or capsule cells could be a defense mechanism to reduce surface area of protonema over which uptake of heavy metals may occur.

The results of Pb and Zn concentrations shows that *Funaria hygrometrica* is a good bioaccumulator of heavy metals (Rao, 1982). The sequestration of heavy metals by the placenta protects the cells during meiosis even in severely polluted environments which increase the ability of *Funaria hygrometrica* to evolve tolerance to heavy metals (Shaw, 1987) and provide resistance of its protonema (Basile *et al.* 1995), probably enhance the ability of *Funaria* to develop and reproduce in the severely polluted areas.
ANTIMICROBIAL ACTIVITY

The antifungal and antibacterial activities of bryophytes have been reported by many biologists. (Madsen & Pates, 1952; Ramaut, 1959). McCleary and Watkinson (1966) reported strong inhibition of gram positive and gram negative organisms by 18 mosses because of its antibiotic properties.

Antibiotically active substances of Atrichum, Dicranum, Mnium, Polytrichum and Sphagnum species are considered to be polyphenolic compounds. Gupta and Singh (1971) observed Barbula and Timmiella species were active against both gram positive and gram negative bacteria. The moss Brachythecium procumbens and the liverworts Asterella sanguina and Marchantia paleacea showed the broadest spectrum of antibiotic activity against the tested microorganisms. Banerjee and Sen (1979) reported that the antimicrobial activity of the specimens depends on its age, season of its collection, physiological state and ecological parameters. In the present investigation, Hydrogonium javanicum and Entodon myurus collected from hilly areas and Physcomitrium coorgense collected from plains show variable activity.

Antibiotic substances seemed to be more frequent in Hepatics than in Mosses and Hornworts. Sabovljevic and Sabovljevic (2008) suggested that this activity of mosses may be due to the presence of various alkaloids present in the mosses. Antimicrobial activity might be due to the presence of flavonoids, steroids, terpenoids and other polyphenolic compounds in the bryophytes (Ilhan et al., 2006).

Prenylbenzyl from Radula species inhibits the growth of Staphylococcus aureus (Asakawa et al., 1982). An acetone extract from the moss Rhyncostegium riparioides and Pleurochaete squarrosa was active on some gram negative bacterial strains (Basile et al., 1998).

Spjut et al. (1986) observed antimicrobial activity in the members of moss families: Thuidiaceae, Mniaceae, Neckeraeace, Hypnaceae, Brachytheciaceae, Polytrichaceae, Dicranaceae and Grimmiaceae and concluded that bryophytes are a source of biological active compounds.

Frahm (2004) tested in vivo antifungal activity of twenty bryophytes on a variety of crops infected with fungi. Ethanol extracts of Bryum argenteum have been
proved to be active against all bacteria and fungi tested (Sabovljevic et al., 2006). Bodade et al. (2008) reported that extracts of *Thuidium*, *Bryum* and *Plagiochasma* shows antimicrobial activity against gram – ve bacteria and *Aspergillus niger*.

Singh et al. (2007) evaluated the antimicrobial activity of extracts of 15 Indian mosses against five gram positive, six gram negative bacterial strains, and 8 fungi. *Sphagnum junghuhnianum*, *Barbula javanica*, *Barbula arcuata*, *Brachythecium populeum*, *Brachythecium rutabulum*, *Mnium marginatum* and *Entodon rubicundus* were found to be most active against all the organisms. Some commonly found bryophyte species with antimicrobial potential are: *Sphagnum*, *Andreaea*, *Atrichum*, *Lyellia*, *Oligotrichum*, *Pogonatum*, *Polytrichum*, *Entosthodon* and *Funaria* (Veljic et al. 2010 ). *Polytrichum commune* exhibited antimicrobial activity (Basile et al., 1999 ; Sharma. et al., 2013).

Traditionally bryophytes have been used for the treatment of different ailments viz. skin diseases, wound healing, inflammation (Tag et al., 2007; Namsa et al., 2009) and viral diseases (Frahm, 2004). According to Asakawa (2007) bryophytes are a rich source of biologically active compounds. Pharmacological investigations have been carried out in different bryophytes to find out their cytotoxic, antibacterial (Basile et al., 1999) and antifungal efficacy (Sabovljevic, 2011).

Alcoholic extracts shows better results than water and other organic solvents (Banerjee & Sen, 1979). Similar observations have been made in the present investigation on *Physcomitrium coorgense*, *Hydrogonium javanicum* and *Entodon myurus*, ethanol extract shows better antimicrobial activity than petroleum ether and aqueous extracts against selected bacterial and fungal strains. The contradiction in the observations of different researchers working on the same plant may be due to different solvents used. Organic and methanol extracts of *E. myurus* were effective against microorganisms used (Kumar & Chaudhary, 2010). In *Physcomitrium japonicum*, out of aqueous and organic extracts, benzene extract is highly effective against microorganisms (Chaudhary & Khanam, 2011). In the present investigation, ethanol extract of *P. coorgense* and *Entodon myurus* have shown antimicrobial activity against all bacterial strains. Contrary to this, *H. javanicum* did not show much antimicrobial activity against various microorganisms tested.
The present study indicates the potential of mosses as a source of antimicrobial phytochemicals. Our observations, in addition to earlier reports, indicate that bryophytes will prove a rich storehouse of drugs not known so far.