DISCUSSION
Control of pests and diseases with the help of insecticidal, fungicidal, acaricidal, antibacterial, antiviral, antihelminthic, anticancer, antifertility, hypoglycaemic, chemotherapeutic, pharmacological products etc. is receiving global attention in recent years (Chopra and Badhwar, 1940; Chopra et al, 1941; Osborne, 1943; Talieva, 1954; Atkinson, 1956; Bell, 1958; Nickel, 1959; Baruah et al, 1964; Bordoloi et al, 1964; Abdullaeva, 1966; Dhar et al, 1968; Bhakuni et al, 1969; Hocking, 1969; Goutam and Purohit, 1974; Sharma and Kulkarni, 1975; Atkinson, 1976; Dhawan et al, 1977; Avadhoot and Verma, 1978; Brahman and Saxena, 1978; Dan et al, 1978; Misra and Chaturvedi, 1978; Rajendran and Gopalan, 1979; Ascher and Walter, 1981; Wilding, 1982; Nath and Bordoloi, 1984; Taneja and Grover, 1982; Mia et al, 1985; Gist et al, 1985; Yinshen et al, 1985; Hussain and Ghulam, 1986; Bodhade et al, 1987; Dutta and Guha, 1988; Karnatak et al, 1990; Dwivedi et al, 1990; Sinha and Gulati, 1990 and Yirdava and Saina, 1990). Likewise, discovery of the therapeutic value of Penicillin and similar antibiotics of microbial origin has stimulated a great deal of interest in exploring the possibilities for finding out such desirable compounds in higher plants. In the recent years, investigation around the world reveals that plant based compounds are likely to play a significant role in the control of many pests and diseases. Even with these compounds, certain invertebrates, such as leeches causing great nuisance to human population are being controlled though this aspect has not been exploited elaborately.
Leeches in general are well known as problematic blood sucking annelids, since their role towards human being are both physical and psychological and they even cause great discomfort to various livestocks. Among the different types of leeches, *Haemadipsa sylvestris* (Blanchard) is commonly found in Assam which causes serious and painful bites when come in contact with desirable warm blooded animals. Protection against such land leeches is an important aspect not only for agricultural and forest field workers, but also for service personnel in military and paramilitary forces placed in various parts of North Eastern Region of India, who have to perform their duties very often in such leech infested areas. *H. sylvestris* is frequently encountered in lower altitudes and foot hills of Assam whereas *H. zeylanica monitivindicis* Moore, another land leech is commonly found at higher altitudes causing great difficulties to human being, particularly for defence personnel.

Considering its occurrence and distribution in different areas with changed eco-climatic situations, an estimation of population density, its frequency and abundance has been made in selected zones covered with different types of vegetation having variable environmental conditions. Vegetation in the area ranges from evergreen to semi-evergreen and to deciduous forests. In case of the low lying areas around "beels" and swamps, a mixed aquatic vegetation with grass land are commonly observed. On the open areas there are mostly scattered patches of scrub forests. The evergreen and semi-evergreen forests on the hilly areas are densely populated by many species of tall trees and lichens. Therefore, in order to have a general view on the distribution pattern of land leeches, three different locations, such as tea gardens, grazing land and Java citronella plantation areas are considered.
where climatic variations due to atmospheric temperature, rainfall, availability of sunlight etc. are more pronounced coupled with varied vegetational structures.

From the experimental observations it is seen that highest frequency of *H. sylvestris* is associated with the months of June, July and August (358.31, 366.64 and 383.32), irrespective of the locations. However, this frequency period varies in different years particularly between June to August. On the other hand, frequency is very less during September onwards to December in both tea gardens and grazing land (191.65 to 208.33, 133.21 to 141.88) while in the areas of citronella plantation, lowest frequency has been noticed during this period, with no record of occurrence of the leeches during January and February and only negligible number of leeches in November and December in both the years. Such is the case with density of land leech, having higher range (106.25 to 105.55) during July in tea garden areas and in August for grazing land (46.41 to 45.74), occurrence of the same is, however, insignificant in citronella plantation. Such variations towards frequency, density and abundance are mainly due to various climatic situations prevailing at different locations of experimental observations. The formation of green canopy of tea bushes due to their periodic prunings and cultivation of shade plants intermixed with tea bushes, may perhaps keep the soil underneath moist, which makes a favourable situation for leeches to breed and multiply by sucking the human blood from tea garden labourers and other workers engaged frequently for various purposes. The intermediate values on frequency, density and abundance in grazing land than that of the tea growing areas, indicated the preference of leeches for moist and shaded
areas where perhaps land leeches may thrive well. In grazing land, which are normally humid with the presence of cattle and warm blooded animals, leeches find their easy means to suck the blood. The distribution pattern in these two localities indicates a prominent variation which may be due to different exposures of sunshine hours because of the changed nature of vegetations. Similar views on distribution and occurrence of land leeches are also expressed by Bhatia and Bora (1973) that shaded and moist areas are more favourable for leeches, even at low and high altitude areas. However, the mild aroma, smoothly emanating from periodic harvested citronella leaves and their ratoon stumps might perhaps develop an unfavourable situation where leeches cannot continue smoothly because of the smell of citronella oil. Further, added advantage are noticed on survival of leeches due to partial shedding by the leaf canopy of the bushes resulting in low population of leeches in such areas.

While comparing with the climatic data, it is further observed that irrespective of the experimental locations, maximum frequency, density and abundance are associated with the temperature range from 25 to 31°C, rainfall from 400 to 500 mm and moderate to high relative humidity of 74 to 92 per cent. The study, however, indicates that in general higher the rainfall, temperature and relative humidity with low sunshine hours, greater is the magnitude of frequency, density and abundance. In-vitro, experiment concerning the effect of temperature on survival of H. sylvestris indicates that temperature upto 34°C suitable for its normal activity beyond which complete mortality is inevitable. Emphasising the effect of temperature on land leeches, Mansfield (1934) observed that this organism could thrive well on any object having temperatures upto 35°C. Similarly, Rajak (1968) reported about the maximum activities of land leech between 25°C and
beyond which shock responses were observed.

Land leeches put to different soil moisture constituents indicates that survival is very poor in soils having varied moisture percentages. However, longevity of leeches varies at higher moisture levels, indicating 55 per cent moisture to be optimum, where the survival period is for 95 days as compared to 6 days with 10 per cent moisture level and 67 days with 100 per cent level of moisture. Such views are also expressed by Saxena et al (1971).

Like other species *H. sylvestris* commences its life cycle from the cocoons of varying sizes with prominent hexagonal cells. The capsules are spherical (0.79 to 1.35 cm dia. and 0.0456 to 0.197 gms in weight), consisting of two parts, i.e. a central mass or embryo which is dark brownish in colour surrounded by a layer of silvery white hexagonal air spaces varying from 29 to 47 in number (mean as 36). Rajak et al (1968) also observe 23 to 40 with 30.8 as the mean hexagonal air spaces in cocoons, whereas Saxena et al (1971) observed 31 to 49 hexagonal air spaces with 42 as mean in the cocoons of such leeches. The slight variations in this aspect may be for locational or regional specificity. The thickness of the air spaces is 0.1 cm on each side and duration after which successive capsules are laid varies from 3 to 10 days. The weights of capsules indicates a regular increase with the time interval, which however, remain unchanged from few days before hatching. The viability of capsules varies from 28.57 to 70.0 per cent while the same is to the extent of 91.9 per cent (Saxena et al, 1971). On the other hand, incubation period seems to be of 14 to 22 days duration with mean as 18.4 which indicates similar ranges of incubation period as reported earlier on land leeches by Rajak et al (1968) and Saxena et al (1971). Hatching of leeches from cocoons is quite common and produces more than two leeches from each capsule with an average of 5.
The freshly hatched leeches in-vitro are fairly active and possess great capacity for retraction and elongation. They are light brown in colour, 0.4 to 2.8 cm in length and 0.0030 to 0.0135 gms in weight. The young leeches resemble their adult ones in their morphological characters with sucking capacity of blood. It is observed that leeches probe the surface of the host body before settling and sucking blood. This period before attachment to the host, i.e. the pre-attachment period is variable for different leeches. On an average, the pre-attachment period, observed from freshly hatched leeches is with a range of 5 to 25 minutes duration and for grown up leeches, the same seems to be 5.5 to 40.5 minutes, showing slight appreciable differences between the age groups. The feeding time in case of young leeches is 10.5 to 65.0 minutes and for the grown up leeches, the time period is 25 to 70 minutes. It reveals that the younger ones have a slight longer feeding time. The ratio of the body weight and the blood taken for young leeches are 1:1.71 to 1:19.9 (mean 1:8.76) and for grown up leeches it is 1:4 to 1:18.6 (mean 1:8.0). This shows that the young leeches have slightly more appetite for blood meal than the grown up ones. Such observations for young land leeches are also recorded by Saxena et al (1971) but with somewhat variable ratios on their body weights. However, the trend of results on body weights between young and grown up leeches was reported to be similar. The general assessment on nutritional requirement for the present leech under investigation indicates that soil substratum is superior for survival in association with the feeding of blood meal. Under the above circumstances H. sylvestris may grow well to attain its maturity from 7 months onward to 9 months approximately. Under constant observations, it is found that the leeches are moving or resting on wet soil surface or attached to the leaves.
of plants throughout the year. However, when the moisture in the surface soil is reduced nearly to dry condition, with the sudden increase in the ambient temperature, it is seen that the leeches will go down in search of wet soil, to avoid dessication and resting during hibernation.

On the other hand, the magnitude of menace created by this organism has led to make search on antileech properties of plants, as this is greatly preferred than that of the synthetic compounds. Williams (1954) reported that an infusion of *Derris elliptica* was highly toxic to leeches. Dixit *et al* (1967) found that oils of citronella, cinnamon (Cassia oil), 1:1 mixture of Acorus oil + haldi oil and 1:1 mixture of Acorus oil + synthetic pine oil was effective on leeches for 5 to 6 hours. Saxena *et al* (1974) reported the importance of insecticidal and repellent properties of *Tephrosia purpurea*, both on land leech and some insects of public health importance. Singh *et al* (1980); Nath *et al* (1982) and Kumar *et al* (1984) studied the commonly available repellents and certain synthetic chemicals against land and water leeches. Earlier, Sai-Leela *et al* (1988) also screened toxicity and repellency of certain North East Indian plants for the land leech, *H. sylvestris* and found that certain plants are of significant importance.

The use of repellents seems to be the best available means for personal protection against annoyance and diseases associated with haematophagous organisms especially because the repellents protect the subject without much disturbing the ecosystem (Painter 1969). However, no such chemicals of synthetic origin and non-hazardous nature are readily available in the market for use by common people at large and not even suitable formulations based on plant constituents are available for the purpose. Now-a-days, plant based
chemicals are more preferable than the synthetics, as the latter are more poisonous in nature, having a risk of contamination of the ecosystem to a great extent due to their persistency and consequent accumulation in the environment (Quadri, 1973). With this view in mind, 42 plants are collected for screening purposes against antileech behaviour. The promising plants showing 50 to 75 per cent and 76 to 100 per cent biological activity are considered for further investigation. They are Acorus calamus, Begonia roxburghii, Cissampelos pareira, Cucumis sativus, Harpullia cupaniodes, Solanum khasianum, Tephrosia purpurea and Entada scandens. On the other hand, the aromatic oils derived from plants like Cinnamomum camphora and C. tamala indicate 50 to 75 per cent repellency, whereas the same from Cymbopogon winterianus, Ocimum gratissimum and Ocimum sanctum shows 76 to 100 per cent repellency.

These variations in toxicity and repellency in preliminary tests lead to conclude that H. sylvestris reacted quite variably to plant based compounds which might be due to the presence of probable active constituents responsible for toxicity or repellency. In order to determine the nature of toxic substances present in the plant tissues, solvent extracts due to petroleum ether, methanol, acetone and chloroform along with aqueous extract also resulted in differential prominence showing specificity of such compounds. The active substance(s) in extracts of Acorus calamus, Begonia roxburghii, Cissampelos pareira, Cucumis sativus, Entada scandens, Harpullia cupaniodes, Solanum Khasianum and Tephrosia purpurea are soluble in most of the solvents. However, the degree of variable toxicity observed from different extracts were perhaps mainly due to the differential solubility of
active substances in respective solvents. It is further noticed that active substances involved in the extracts are mostly soluble in water and to a certain extent in some of the solvents. Chemical investigation made in this direction indicates that saponin fraction has played a more dominant role in toxicity to land leech than that of the alkaloid components supporting the views expressed by Guruswamy and Amala Bhaskar (1971) that glyco-alkaloidal fractions of *Solanum trilobatum* did not give any appreciable effect on leeches. Banerjee and Ganguly (1976) also reported that alkaloid component of *Solanum khaslanum* was less effective on *Hirudinaria javenica*. On the other hand, better results on toxicity is observed with saponin component which are widely distributed in many of the plants. This is mostly soluble in water but not so in ether etc. They give a soapy foam when dissolved in water and are reported to be toxic to cold blooded animals (Tschese and Wuft, 1964; and Vosel, 1963). The toxicity observed in this present investigation by the above plant constituent is perhaps due to saponin part as the concerned plant extracts have responded positively for presence of saponin, confirming the same for present investigation as well (Kapoor, 1969, 71 & 75 and Kumar et al, 1990). To obtain more data on this aspect, the study on *Solanum khaslanum* extract reveals that saponin part is more active towards toxicity than the alkaloid solasodine extracted from the berries of *S. khaslanum*.

On the other hand, many of the aromatic oils even at their diluted concentrations indicates strong repellency on *H. sylvestris*. Such activities due to various aromatic oils are previously reported in case of other organisms like fungi, bacteria, nematodes etc. Dwivedi et al (1990) reported the fungitoxicity of ten different essential oils, where *Lippia alba* exhibited the
absolute inhibition of mycelial growth of *Macrophomina phaseolina* at 2000 ppm. Sinha and Gulati (1990) observed antibacterial and antifungal properties of aromatic oils from *Ocimum* species viz. *Ocimum sanctum* and *Ocimum basilicum* and reported that 1:1 combinations of the oils were more effective than the individual oils. Golke (1990), worked on nematicidal activity of *Myristica fragrans* against *Meloidogyne incognita* and found that the acetone extracts of aerial part was more potent. Raghavalah and Jayaramlath (1988) screened Lemongrass, Palmarosa, Geranium and *Eucalyptus citriodora* oils against the muscardine, *Beauveria bassiana* (Bals) Vuill and observed the antifungal activity of these oils as disinfectants of silkworm rearing equipments. The essential oil of *Eupatorium triplinerve* leaves indicated considerable antimicrobial efficacy towards many bacteria and fungi (Yrdava and Saini, 1990) Yinshen *et al* (1985) isolated and identified the mosquito repellents from *Artemesia vulgaris*, which were mostly due to linalool, camphor and a number of monoterpenoid compounds.

The oils of *Ocimum gratissium*, *Ocimum sanctum* and *Cymbopogon winterianus* show high repellency against *H. sylvestris*, whereas the oil of *Cinnamomum* spp. indicates only moderate repellency. Dixit *et al* (1967) also observed that Citronella and Cinnamon (Cassia) oils in association with some other products produced repellency on land leeches upto 6 hours, but specificity of compounds from such oils was not elaborated in their studies. However, the probable constituents of these oils were studied using GLC technique and identified that both the *Ocimum* species contained eugenol (69-75%) to a great extent whereas *cinnamomum tamala* contained only 53.2% eugenol. The oil of Java citronella (*Cymbopogon winterianus*) contained citronellal (36.4%), geraniol (25.0%), geranyl acetate (8.9%), citronellol
(6.2%) and citronellyl acetate (5.6%). On the other hand, *Cinnamomum camphora* contained 68.1 per cent cineol. To assess the biological activity of these oil based chemical constituents for repellency of *H. sylvestris*, it was further studied with known concentrations of pure compounds which revealed that eugenol, citronellal and citronellol showed the highest activity followed by geraniol and cineol, whereas geranyl acetate and citronellyl acetate indicated comparatively a low status of repellency. Although eugenol seemed to be a major constituent for repellency of *H. sylvestris* but differential behaviour of repellency due to three eugenol containing oils from three different plants could be for their varied proportions in the respective oils. Certain aromatic plant constituents, repellent to land leeches were emphasised earlier by Dixit et al., (1967) and Nath et al., (1986). The repellent effect of citronella oil on certain insects was also reported by Sarawathi and Rao (1987). Hence, the experimental findings suggest that some of the plant based compounds may profitably be used against toxicity and repellency of *H. sylvestris*.

Storage of the plant extracts and the distilled oils considerably effect in their potency on repellency, whereas the fresh samples had high repellent properties (Gulliver, 1947; Sehgal, 1961 and Dixit et al., 1977) as compared to old samples. Many of the aromatic and volatile plant components were lost during storage leading to lower activity of the products. Similar views on storage of essential oils were expressed by Sinha et al., (1990) when they studied the composition of essential oils in the peel of Indian Mandarin oranges (*Citrus reticulata* Blance) and found that the oil composition changed during storage upto 42 days.
It thus appears that although a large majority of plants are available around Jorhat area, all of them are not relevant from the point of view of antleech properties. However, investigations in this line to identify various constituents responsible for toxicity and repellency of *H. sylvestris* from many other plant extracts may significantly enhance the utility of plant based constituents. It is also observed that apart from *Acorus calamus*, *Begonia roxburghii*, *Clissampelos pariera*, *Cucumis sativus*, *Harpullia cupanoides*, *Solanum khasianum*, *Tephrosia purpurea*, *Entada scandens*, *Cinnamomum camphora*, *Cinnamomum tamala*, *Cymbopogon winterianus*, *Ocimum gratissimum* and *Ocimum sanctum*, there are number of plants which indicate moderate or partial toxicity and repellency. Hence, to explore more on this aspect, further investigation is necessary to elucidate other factors concerned with different plant extracts.