A large number of plant products have been traditionally used for protection of grains in storage in India. The validity and advantages of such traditional uses have been examined in a large number of cases. In case of rice, leaves of a locally available plant known as begunia, *Vitex negundo*, have been tested by carefully planned experiments (Prakash, 1980; Prakash et al., 1981e). Similarly leaves of the plant locally known as bel, *Aegle marmelos* (Prakash, 1981; Prakash et al., 1982b) and leaves of wild sage, *Lippia geminata* (Prakash and Rao, 1983) have also been found useful in paddy storage. However, begunia leaves offered greatest protection (Prakash et al., 1982a).

The limitations for the use of plant products as such in grain storage are well known, particularly to be of use in large scale storage. Equally there is a group of advocates who would like to see a total ban on the use of synthetic chemicals particularly admixture with the grains for their protection during storage. It, therefore, calls for detailed investigations on plant products so as to get the active components in plant products rather cheaply and use them with advantage. Such attempts were made as early as 1972 (Barton, 1972) against *Tenebrio molitor* a pest in wheat flour. However, such attempts in case of storage insects of rice are wanting. The present investigations were, therefore taken up with begunia and
bel leaves. The latter plant had to be given up in view of high cost involved in extracting adequate quantity of the active component for studies.

The investigations involved extraction, isolation and identification of the active principles/components, testing the effectivity and mode of action against pre-selected test storage insects and study the safety for their use.

5.1. Isolation

Earlier the extraction of plant materials was attempted by steam distillation, maceration and extractions with solvents and enfleurage (Dethier, 1947). These days the widely accepted and an appropriate method of extraction is by solvents using column chromatography and thin layer chromatography. In the present studies the latest methods were adopted and the extracts and fractions obtained were tested for biological activity (toxicity/repellency). This was measured as a function of number of eggs laid, larvae hatched and adults emerged of the test species in grains treated with the extract or fractions and comparing it respectively with solvents used for extraction or fractionation. The fractions which possessed biological activity were processed further till chemically pure active component was obtained for its chemical identification.
Tests were undertaken for its efficacy and safety when used under laboratory conditions and under natural conditions so as to find its utility in an insect pest management programme.

During the process of evaluation of biologically active extract/fractions in the process of its isolation, it was observed that majority of the test insects died after egg laying in case of grains treated with respective solvents used for extraction/fractionation. In case of grains treated with biologically active extract/fractions the test adults were found restlessly moving on the grains for a period of 10 to 30 minutes and then moved to the grains treated with respective solvents and died after egg laying. During the period of restlessness the females of the test species laid a few eggs on the grains treated with the biologically active extract/fractions also but the fecundity was significantly reduced when compared with the fecundity of these females on the grains treated with respective solvents. Thus it appeared that biologically active extract/fractions coated on the grains acted as a repellent, which inhibited the egg laying of each test species and as a consequence of which the hatching of larvae and emergence of adults reduced. The biologically active extract/fractions did not show direct inhibition in development or mortality in larval hatching and adult emergence. This was separately confirmed in two laboratory experiments for hatching of
larvae and pupation of the test species under the process of laboratory evaluation of the active component (Tables 18 and 19).

Repellents are those substances which as stimuli elicit avoiding reactions for organisms. These are categorised as physical repellents like morphological structures of hosts i.e. hairs, spines, rough surfaces of leaves and husk etc. and as chemical repellents like chemical constituents of host plants or animals secreted or coated on their body surfaces (Dethier, 1947). According to Shorey and John (1977) insect repellents are the chemicals which act in vapour phase and prevent insects for reaching the target to which they otherwise are attracted. Chemical repellents are further divided in two categories namely volatile repellents, which tend to act as olfactory repellents and the other non-volatile repellents which act as gustatory repellents. Gustatory repellents are generally responded by taste organs and ovipositors of the insects. As a result the adults show avoiding reactions to these substances. The biological activity of the active extract/fractions under the present study appeared to be that of a repellent to the test insects, which was confirmed by the various laboratory tests conducted for evaluating the active component.
5.2. Identification

Plant origin chemicals of ketonic group have so far not been reported as grain protectants against storage insects. The active component isolated from begunia leaves in the present study is an open chain of ketonic compound of \( C_{37}H_{74}O \) molecular formula. Earlier from another species of Vitex i.e. \textit{V. megapotamica}, phytoecolsoids viz., viticosterone-E, iridoides and ecylones have been isolated and identified (Rimpler and Schulz, 1967; Rimpler, 1969, 1972). These chemicals are reported to affect the moulting of insects.

There is a common belief with the farmers who use begunia leaves as rice grain protectant that due to typical aromatic smell of begunia leaves the storage insects do not infest the grain. The characteristic smell was found only in fraction II-1(2), a yellowish red oily component. Interestingly in this fraction, which was discarded, showed biological activity in case of \textit{S. oryzae} eggs only. As expected no activity was exhibited against the larvae and adult emergence. Since this fraction did not show activity against all three test insect species as was the case of fraction II-1(1), the fraction II-1(2) was discarded for the present studies. The possibility of a narrow spectrum activity in fraction II-1(2) needs to be further explored.
5.3. Evaluation

Laboratory tests: Under laboratory tests, various concentrations of the active component were tested for quantifying its dosage to be used for testing under natural conditions of insect infestation. The concentration at which no egg laying took place in laboratory and as a result of which further development of larvae and emergence of adults of the test insects were completely checked, varied with insect species. It was found above 200 ppm in case of *S. cerealella*, 300 ppm in case of *R. dominica* and 400 ppm in case of *S. oryzae*. Effectiveness of the isolated component was highest against *S. cerealella* followed by *R. dominica* and *S. oryzae*.

To study the mode of action of the active component under laboratory conditions, different tests were performed. The active component was found to inhibit the egg laying of the test insects (Table 21) and did not directly inhibit significantly larval hatching and/or pupation of the test insects. This was confirmed in two separate tests (Tables 18 and 19). Tests were also performed to study the effect of the active component on the behaviour of test insects in terms of restlessness, stay-in-period and longevity of adults under both free choice and confined conditions. Longevity of adults was found to be
unaffected by the active component. Adults were restless on the grains soaked with the solutions of the active component and stayed for lesser period on these grains when compared to the grains soaked with only solvents (controls) under free choice conditions (Table 20). Further, when the active component solutions were applied topically to the adults of test insects using Potter's Tower, it did not significantly affect the mortality of adults of test species, though fecundity of such adults reduced significantly (Table 22).

Based on these laboratory tests it was concluded that the active component only affected/inhibited the egg laying of the adults and showed no direct adverse effect on larval hatching, pupation, adult emergence and longevity of adults. The reduced adult emergence and grain damage actually results from reduced egg laying. The adults expressed avoiding reactions of not staying on the grains treated with the active component for a period for which they normally stay for egg laying on untreated grains. In addition they migrate to the grains treated with only the solvents under free choice conditions. These data and results conclusively prove that the active component acts as a repellent. Dethier (1947) and Shorey and John (1977) stated that volatility of a chemical was a pre-requisite for an olfactory repellent. The presently identified active component is a non-volatile and odourless
compound. As such it is believed that this substance acts as a gustatory repellent. The reduction in egg laying of test insects can be explained as a function of gustatory repellency.

Tests under natural storage conditions: Insect repellents have been grouped under the category, Insect Behaviour Regulators (IBRS) and have been used in pest management of agricultural crops and stored products (Young and Silverstein, 1975). In order to assess the utility of the active component isolated in the present study it was tested under natural storage conditions using 50 and 400 ppm concentrations both in gunny bags and tin structures (a simulated condition for bulk storage) for a period of 270 days. The component was evaluated for the grain damage by S. cerealella, R. dominica and S. oryzae. The concentrations at which oviposition on treated grain by test species was completely checked in laboratory tests, did not protect the grains completely from storage pests in these tests under natural conditions. Even at highest concentration of 400 ppm of the active component, grains were damaged in both structures and nearly 50 per cent grain protection was achieved for 225 days as compared to control (Tables 27 and 28). In laboratory tests at constant temperature (28 ± 1°C) and relative humidity (85 ± 2%) maintained for a period of 9 months, the effectiveness of the active
component was found to be unaffected with the passage of time (Table 23). This is normally expected of a non-volatile repellent compound. However, the effectiveness of the active component was found diminishing with the increase in storage period in the natural conditions of storage (Figs. 13 and 14). It is possibly due to the existence of high relative humidity and temperature during the natural storage. These factors have been considered as detrimental and diminish the effectiveness of a grain protectant used in storage (Abdel-Kader et al., 1982; Prakash, 1983a).

A successful grain protectant should not affect adversely the viability of grains which are treated (Elliott et al., 1978). The active component in present studies did not affect grain viability adversely both at 5th and 14th day of plating of the grains treated with 50 and 400 ppm concentrations of the active component and with respect to solvent i.e. hexane and water throughout the storage period (Tables 30 and 31). Viability significance was found to be positive (Table 32), which may be because of less insect damage in the grains treated with the active component compared to the grains treated with hexane or water.

Swelling index (dry basis) in terms of water uptake and optimum cooking period are considered as parameters to study the cooking quality of grains (Mohsenin, 1970).
FIG. 13. PER CENT GRAIN DAMAGE AT DIFFERENT STORAGE PERIODS DUE TO S. CEREALELLA AND R. DOMINICA STORED IN BAGS AND IN TIN STRUCTURES.
FIG. 14. PER CENT GRAIN DAMAGE AT DIFFERENT STORAGE PERIODS DUE TO S. ORYZAE AND TOTAL % GRAIN DAMAGE STORED IN BAGS AND IN TIN STRUCTURES.
Results showed negligible differences between water uptake and optimum cooking period and also between swelling indices of treated and untreated (controls) rice kernels with the active component at both 180 and 270 days of storage in bags and tin structures. Thus the active component did not adversely affect the cooking quality of grains even when treated at 400 ppm concentrations.

No adverse effect was observed when dermal, oral and acute mammalian toxicities were tested on albino mice. \( \text{LD}_{50} \) were found as high as 5625 mg/kg body weight in female and 5490 mg/kg body weight in male mice which were much higher than chemicals like malathion \( \geq 1600 \) mg/kg body weight (Anon., 1965) and etrimfos \( \geq 1800 \) mg/kg body weight (Anon., 1980), which are presently being used as grain protectant in storage.

The active component isolated in the present study is safe and effective for use as grain protectant against the three test insect pests attacking rice in storage. A cheap and effective method of extraction of the compound commercially or synthesizing the compound has to be attempted.