Karnal bunt of wheat was first observed at Karnal in 1930. The fungus causing the disease being morphologically different from the other known bunt fungi recorded on wheat was named by Mitra (1931) as *Tilletia indica*. Mundkur (1940) after a restudy of the morphology and germination of teliospore, renamed it *Neovoossia indica*. Duran and Fischer (1961) prefer the name *T. indica* because in their opinion the criteria employed by Mundkur for transferring this fungus to *Neovoossia* were not sound and are shared by many species of *Tilletia*. They cited two species of *Tilletia* (*T. elymii* and *T. cerebrina*) which have multinucleate sporidia that do not fuse to form a shaped connections. Siang (1954) remarked that in contrast to dicaryons of *T. cerebrina* and *T. elymii*, the solapathogenic lines of *Ustilago maydis* are culturable. The binucleate primary sporidia of *Neovoossia indica* are also culturable and thus biologically distinct from *Tilletia* species. Morphological and cytological studies reported in this work confirm the observations of earlier workers in this regard. However, some additional information on spore formation and nuclear condition of the fungus observed in the present investigation clearly showsthat it is distinct from *Tilletia*. The application of the name *N. indica* is, therefore, preferred.

The study of physiology provides a better understanding of the functions or life processes of an organism. Ramamooorthy and Mundkur (1944) established culture of *Neovoossia indica*
on potato dextrose agar and Holton (1949) recognised 8 cultural types of colonies on the same medium. However, for determining the nutritional requirements of the fungus, the selection of a synthetic (chemically defined) medium is essential.

In the present study Czapek's plus yeast extract medium was found to be the most suitable for growth and sporulation of *N. indica*. In a preliminary study the need for yeast extract became obvious. Several workers have indicated the necessity of yeast extract for good growth and sporulation of fungi as in case of *Cercospora personata* (Shanta, 1956); *G. viticola* (Sethi and Munjal, 1963); *Phycomyces blakesleeanus* (Schopfer, 1935); *Alternaria solani* (Lewis, 1952) and *Coprinus lagopus* (Madelin, 1956). Effect of different concentrations of yeast extract showed that the growth of the fungus goes on increasing with increase in the concentration up to 1 g/l and then starts falling at higher concentrations. Yeast extract is a good source of B group vitamins and some amino acids. In the vitamin requirements, the fungus shows a deficiency for thiamine. Schopfer and Blumer (1938) on the basis of vitamin or other growth factor requirements of eleven *Natalago* species, classified them as auxosaprotrophic or auxoheterotrophic. The latter being the ones which are dependent upon growth factor. *N. indica* belongs to this category. Blumer and Schopfer (1940) found that the components of aneurin molecule, pyrimidine and thiazole were necessary for the growth of *Tilletia horrida*. 
In the present studies, the temperature of 20°C and reaction of the medium at pH 5.5 appear to be optimum for the growth as well as sporulation. Ramamoorthy and Mundkur (1944) reported that *N. indica* grows best at 18°C. It is probable that different isolates of the fungus may behave somewhat differently. Similar observations were made for *Ustilago nuda tritici* by Sartoris (1924), Sen and Munjal (1968) and Skvortzoff (1938) who got the best growth of this fungus at 30°C, 25°C and 22-24°C respectively. In pH requirement *N. indica* agrees with the findings of Wolpert (1924) who reported that pH optimum of most Basidiomycetes lies in the neighbourhood of 5.5. Sartoris (1924) observed that a slightly acidic medium was optimum for the growth of *N. heufleri*.

Various constituents of the basal medium were scrutinized as regards quantity in relation to their utilization by the fungus. Reduction of the quantities of sucrose and nitrogen below the normal level of the medium sharply reduces the dry weight of *N. indica*. Higher yields of dry weight result at higher concentrations of sucrose but the sporulation is reduced. In the case of sodium nitrate both growth and sporulation are reduced. Magnesium sulphate and potassium chloride have little effect. It is very likely that the fungus is able to utilise some of the potassium present in the Sorensen's buffer, which had been added in all the experiments on nutritional requirements. Because of buffer, the requirement of phosphate also could not be determined. Ability to utilise different carbon sources by
the pathogen depends on its enzymatic make up. Best growth of *N. indica* occurs on glucose followed by sucrose and fructose. Maltose, a disaccharide, has been very popular as a carbohydrate source in the culture of smut fungi. Sartoris (1924) found its superiority over other carbohydrates for various smut fungi and Thren (1937) found it excellent for the culture of haplonts of *Ustilago nuda*. On the contrary, maltose supports poor growth and sporulation of *N. indica*. Sen and Munjal (1968) got similar results with *Ustilago nuda tritici*. The other two disaccharides galactose and lactose also do not support good growth of *N. indica*. The polysaccharides, inulin and starch are poorly utilised. They support less growth and scanty sporulation. Starch consists of repeating units of maltose. Only those fungi which produce the enzyme amylase are able to utilise starch. This indicates that *N. indica* does not produce amylase. Of the three sugar alcohols tried, mannitol proves to be superior for growth of *N. indica*. Its sugar mannose supports better growth than itself but both support scanty sporulation. *N. indica* does not produce any sporidia on sorbitol.

Alanine, asparagine, aspartic acid, arginine, glutamic acid and peptone support good growth and sporulation of *N. indica*, than sodium nitrate. This supports the view of Fischer and Holton (1957) that inorganic sources of nitrogen are inferior to the organic sources for culturing the smut fungi. *N. indica* gets its nutrition from the developing grain. It is fair to assume that organic nitrogen will be more in this plant tissue.
The disease was earlier reported to occur in some submontaneous districts of the Punjab where it is considered to be endemic. The survey conducted during the last two years has shown that the disease is now occurring over a wider area. This is due to the favourable environmental conditions for the development of the disease. Although all the commercial wheat varieties of India have proved to be susceptible to this disease in the varietal resistance tests, the large scale cultivation of Mexican wheats in which flowering is uniform and early as compared to other Indian commercial wheats coupled with high doses of nitrogenous fertilisers are also responsible for the distribution of the disease over larger areas.

The development of a disease in epiphytotic proportions is dependent on the congruence of three factors namely abundant viable inoculum, cultivation of susceptible host over vast areas and favourable environmental conditions. Karnal bunt appears in some years in serious form. Thus the presence of large amount of viable teliospores and conditions favourable for their germination would ensure abundant inoculum potential. Studies made on germination of teliospores indicate that presoaking of spores result in better germination. Similar observations were made by Mundkur (1943), Bedi et al. (1949) and Holton (1949). Presoaking was found to be useful for germination of spores of *Urocystis tritici* by Noble (1924) and of *Uncinula striiformis* by Thirumalachar and Dickson (1947). Spores floated on water show the maximum germination.
of *N. indica*. Davis (1923) observed that after ripened spores of *U. striiformis* germinate either floating or submerged in distilled water but do not germinate when placed in damp surfaces. The germination is more in sterilised water. This is probably due to containing fungi present in the unsterilised water especially *Alternaria tenuis* and *Penicillium* spp. which exerted an inhibitory influence. Tap water gave better germination than distilled water. This supports the observation of Mitra (1933). The spores germinate over a narrow range of temperature of 10–25°C with optimum at 20°C. These results vary slightly from that of Mitra (1931) who reported no germination at 10°C. This difference can be attributed to the effect of presoaking of spores. It is further noticed that spores can germinate over a wide range of pH 5–9, with optimum at pH 6. Many workers have found acidic medium better for the germination of spores, as in the case of *Urocystis occultae* and *Ustilago coica* for which the optimum pH 6.6 and 6.4 was reported by Ling (1940) and Chowdhury (1946) respectively. Johnson (1931) and Prasada (1948) observed that teliospores of several rust fungi formed under different environmental conditions show marked difference in their germination. Spores of *N. indica* collected from different localities do not show any appreciable difference in germination. Similar observation was also made by Mundkur (1943). The spores of *N. indica* germinate equally well in light and darkness. Baylis (1955) observed that light stimulated germination of
spores of *Tilletia controversa*, the dwarf bunt organism, where optimum germination occurred at approximately 5°C under constant light. Germination seldom occurred in darkness. Ling (1940) on the other hand found darkness or diffused light best for spore germination in *Urocystis occulta*.

Since water is a universal solvent, under natural conditions, the spores do not come in contact with pure water. The beneficial effect of tap water over distilled water has already been reported here. Various nutrients such as sucrose, glucose, organic and inorganic nitrogenous compounds do not show any marked increase in the percentage germination of spores of *N. indica*. There are contradictory reports about the role of nutrients in stimulating spore germination of fungi and it may vary with the type of the fungus in each case.

The sugar solutions exerted a favourable influence on spore germination was noted for *Cercospore sesami* Zimm. and *Ustilago coccae* by Chowdhury (1944, 1946), for *Ustilago tritici*, *U. hordei* and *U. zea* (Stakman, 1923) and for *Glomerella cingulata* by Lin (1945). Amino acids and vitamins were found to increase germination of spores of *Colletotrichum gloeosporioides* by Cooper (1939). It was, however, noted by Lin (1940) that conidia of *Sclerotinia fructicola* did not require any energy source for germination. Davis (1923) stated that nourishing solutions and decoctions are of no value in forcing germination of *Ustilago striiformis*. Mitra
(1935) also made similar observations for *Neovossia indica*.

It is well known to all students of fungi that spores of some fungi would germinate as soon as released from their parent cells (sporulates). Teliospores of many smut and rust fungi and oospores and zygospores of lower fungi, however, require a rest period. This is probably a biological necessity because during this period their natural hosts are absent in the field. Notable among spores that require a rest or dormant period are those of *Ustilago striiformis* (Davis, 1924), *Urocystis tritici* (Noble, 1923), *Tilletia controversa* and *Puccinia graminis* (Stakman and Harrar, 1957). This is a boon to the organism, as it helps in its perpetuation. The studies on the viability of *Neovossia indica* show that the fungus belongs to this category as the spores cannot germinate earlier than six months. This is in agreement with the observations of Mitra (1935), Mundkur (1943) and Vasudeva (1957).

In the present studies, teliospores of *N. indica* show viability for four years. These results confirm the findings of Mathur and Ram (1963). Fischer (1936) in a study of herbarium specimens reported that a number of smut fungi were found to retain their viability for long periods: 25 years (*Tilletia levis*), 18 years (*T. tritici*), 23 years (*Ustilago hordei*), 13 years each (*U. avenae* and *Sphaerolotheca sorghi*).

Storage conditions of spores may also affect their viability. There was not much difference in the spores kept in the laboratory or these kept in the field but spores
stored in the refrigerator retained their viability for a longer period. This is true for most fungi (Lambert, 1929; Johnson, 1941; Tapke, 1948).

Freezing and thawing or exposing the spores to heat treatment for short periods do not have any effect on the germination of teliospores of *N. indica*. Several workers have used certain stimulants to get good germination of smut fungi. Johnson (1931) was able to get germination of *Puccinia graminis* by freezing and thawing. Goddard (1935) induced the dormant spores of *Neurospora tetrasperma* to germinate by heat treatment. Thus response of such treatments may vary with different organisms.

Enomoto (1934) reported a stimulating effect of ether on spore germination in several species of *Ustilago*, *Sorosporum* and *Sphaeclotheca*. Exposing the spore of *N. indica* to vapours of ether, or acetone does not show any stimulatory action though exposure to chloroform has slight adverse effect. Benzaldehyde treatment of spores has been found useful by Noble (1924) for *Urocystis tritici* and for *N. occultans* by Ling (1940). This treatment does not help in spore germination of *N. indica*. Thirumalachar and Dickson (1947) also did not find it satisfactory for spore germination of *Ustilago striiformis*. Stimulation in the germination of uredospore of wheat stem rust by terpenes and related compounds was reported by French (1961) but the spores of *N. indica* do not respond to treatment with alphapinene. Treatment of spores with citric acid was found useful by
Thiel and Weiss (1920) for *Puccinia graminis tritici* but this induces slight stimulation.

Presoaked spores of *N. indica* when treated with potassium permanganate show slight stimulation in germination. Thirumalachar and Narasimhan (1953) found it satisfactory for several fungi. Cheo and Leach (1950) observed stimulating effect of dung infusion on germination of spores of *Jatilago striiformis*. Cowdung extract shows some stimulatory effect in case of *N. indica*. Soil extract increases spore germination of *N. indica* slightly but Mathur and Ram (1953) got 61.4 per cent germination in this medium. Mitra (1935) did not find any stimulation with this treatment.

Host plants, pieces of host plants and the expressed juices from plants may stimulate germination considerably (Brown, 1936). Root extracts taken at the flowering stage of the plant from a susceptible wheat variety, does not show such effect but grain extract taken at the dough stage stimulates spore germination of *N. indica*. This may be due to the presence of some nutrients present in the grain extract.

Vitamins and amino acids do not markedly stimulate spore germination of *N. indica*, although they exert a good effect on growth. Ryan (1948) reported that amino acids favoured spore germination in mutants of *Neurospora* species deficient in these amino acids.

Teliospores of *N. indica* placed in a clay soil germinate better than in sandy soil. The germination is
further stimulated by addition of farm yard manure.

Mundkur (1943) definitely established that sporidia produced from the germinating spores of *N. indica* became air-borne and infected individual florets. The direct proof of this phenomenon is shown by trapping the sporidia on rod traps.

Sporidia produced from the germinating spores are relatively more delicate than spores. They must germinate to infect the host. It has been shown that sporidia of *N. indica* germinate at a temperature range of 10–25°C, with optimum germination at 20°C. The sporidia do not germinate at relative humidity (R.H.) of 30 per cent but germination increases with the increase in R.H. The sporidia exposed to 30 per cent R.H. for 36 hours totally lose their viability. This explains why the disease is more prevalent in relatively wet years when the R.H. is more and temperatures are moderate. Both these factors operate together.

No information is available on the mode of infection of *N. indica*. The histopathological studies show that the fungus enters the developing grain from the glumes through the ovary wall. This is also corroborated by detecting the radio activity of labelled inoculum found in the glumes and ovary four weeks after inoculation.

In the infection studies of *N. indica* inoculum consisting of 99 per cent primary sporidia shows maximum infection followed closely by inoculum with secondary sporidia. Mycelial suspension produces very low infection.
Tisdale and Johnston (1926) working with corn smut fungus \( \text{Ustilago maydis} \) observed that old cultures became mycelioid and less virulent. Inoculum consisting of spore suspension also results in poor infection. It appears that spores of \( \text{N. indica} \) which take about two weeks to germinate, cannot produce high infection because by then a large number of grains would have passed the susceptible stage of infection.

Low concentrations of inoculum produce low infections of \( \text{N. indica} \). Similar results were reported by other workers dealing with \( \text{Ustilago bullata} \) (Fischer and Holton, 1957) and \( \text{G. nuda tritici} \) (Chatrath and Bahl, 1966).

The present results show that isolates of \( \text{N. indica} \) vary considerably in their virulence. Such a behaviour is well known in practically all pathogenic fungi.

Environmental factors govern the development of diseases is well known. Maximum disease incidence of \( \text{N. indica} \) is observed when the air temperatures vary between 10–24°C with relative humidity at 82–100 per cent. Mundkur (1943), Bedi et al. (1949) and Sattar and Hafiz (1952), on the basis of field observations, stated that low temperatures and high humidity at the time of ear formation were conducive to development of the disease. Mundkur (1943) and Bedi et al. (1949) also correlated it with the rainfall at the flowering time but did not give any specific indication of the temperature and relative humidity. The observations made in the present study are supported by the earlier results.
of germination of sporidia. When there is more variation in temperature range of relative humidity especially during the 48 hours following inoculations, the pathogen does not appear to establish itself and therefore either nil or low infections occur. Tapke (1929, 1931) found that infection of *Ustilago tritici* was much less if the relative humidity of the atmosphere was low at the time of flowering of wheat. Chowdhury (1951), on the basis of field observations, indicated that development of bunt (kernel smut) of rice was influenced by climatic conditions particularly by occurrence of frequent light rain fall and humid weather when the plants were in flower stage. Padwick (1939) and Bedi et al. (1949) observed that heavy irrigation of fields increased the disease incidence. This would indirectly increase the relative humidity of the crop and make it more vulnerable to the attack of the pathogen.

Fertilizer application results in higher incidence of *N. indica* except where a very heavy dose of phosphorus was applied. Maximum infection occurs in treatment having lowest dose of phosphorus, followed by treatments with nitrogen. Bedi et al. (1949) observed that heavy manuring results in higher incidence of the disease. Templeton (1963) reported that maximum infection of kernel smut of rice occurred on highly fertilized soils. The results obtained in the present experiment cannot be considered conclusive as the same treatment ($N_{60} F_{40} K_{40}$) shows variable infections. Holton and Heald (1941) have reviewed the work done on application of fertilizers for the control of wheat bunt caused by
Tilletia caries and T. foetida and remarked that the conflicting reports from different investigators do not permit definite generalizations.

It has been observed that when the temperatures start rising, the grain formation is quicker and then little or no infection of *N. indica* may occur or where it has taken place the further growth of the fungus is arrested. This is depicted in the typical symptoms produced in this disease where partial smutting of the grain is often the rule. In order to see if any chemical would slow this process and thus help in better understanding of the pathogenesis, maleic hydrazide was sprayed at two concentrations namely 80 and 160 ppm on the susceptible variety HP 720. Best response is noted at 160 ppm dose, which resulted in much higher infection. Maleic hydrazide is known to serve as inhibitor of growth by several workers (Currier et al., 1951; Compton, 1952; Naylor and Davis, 1951; Esato, 1957, etc.) and also as a promoter of the severity of many diseases like wilt, rusts and leaf spots (Simons, 1955; Richards, 1957; Nair, 1958 etc.). All varieties do not respond the same way to the chemical (Samborski and Shaw, 1957, Simons, 1955). This differential response was also confirmed in the present studies.

According to Stakman and Harrar (1957) there is abundant evidence that fungi usually penetrate young and tender plant parts. Thus the ovaries of wheat and barley are susceptible to penetration by loose smuts for only a
few days to a week, the coleoptiles of seedlings are penetrated by certain smuts only when the seedlings are young. It has been shown by Chona et al. (1961) that infection of *M. indica* takes place when sporidia are injected into ear while still enclosed in the boot leaf as well as by Moore's partial vacuum method at the time of anthesis. Studies made to find the most susceptible stage for infection have shown that maximum infection occurs when the awns were just emerging out of the boot. It becomes less as the boot starts swelling. Least infection occurs at the preanthesis stage. It shows a rise again at the anthesis or post anthesis stage but completely drops at the dough stage. This could be due to the availability of some metabolites which are necessary for the development of *M. indica*. Moore (1936) reported that midanthesis was most susceptible stage for loose smut of wheat.

The host range studies show that this pathogen is restricted to wheat alone. This is supported by the field observations, as no other grass or cultivated crop except wheat has been found infected by *M. indica*.

The control of the fungus was aimed at eliminating the seed borne infection as soil borne infection is difficult to deal. If we can successfully control the seed borne infections it will minimise the chance of spread of the disease to new areas through infected seed. When the spores were put in different fungicidal solutions, they failed to germinate except at 500 ppm concentrations of
Plantvax (P461) or Vitavax (D735). This establishes the toxicity of the fungicides to teliospores of *H. indica*. The oxanthin compound Vitavax has shown promise for the control of loose smut of wheat and barley (Edgington and Heinberg, 1966; Chatrath et al., 1969). It has become a practice in India that some of the seed producing agencies like National Seeds Corporation, leave a packet of fungicides in the seed bag so that the farmer can use the same for dressing the seed before sowing. These fungicides include Ceresan and Agrosan, the organomercurials which are known to act on the seed through the vapour action also. New Improved Ceresan and Ceresan completely inhibit spore germination through their vapour action but had slight adverse effect on seed germination. The remaining six fungicides give only a partial control of the pathogen. When these fungicides are applied directly on the seed surface at their normally recommended doses, the treatment is slightly less efficacious. The difference may be due to the quantity of fungicide used by the two methods. These two experiments clearly lead us to the conclusion that seed treatment with fungicide does not completely eradicate the seed borne infection. Beneficial effect of fungicidal seed treatment in eliminating seed borne infection in several smut fungi has been reported by numerous workers (Yu et al., 1934; Holton and Woo, 1952; Fischer and Meiners, 1952; Leukel, 1948; Purdy, 1955, etc.).

Since the discovery of hot water treatment by Jensen
(1888) for the control of embryo infecting smut of wheat and barley, this method has been successfully employed by several workers for the control of several other fungal and bacterial plant pathogens (Cook, 1941; Dickson, 1956; Walker, 1969; Srivastava and Rao, 1963). In the present investigation, direct immersion of seed in hot water at 54°C for 10 minutes completely eliminated infection without much adverse effect on seed viability. In the seed presoaked for 5 hours and then subjected to the same treatment, one per cent of the spores still germinated. This may be due to some physiological change in the spores on account of presoaking. Mitra (1935, 1937) showed beneficial effect of seed treatment with fungicides and hot water (Mitra, 1937) for the control of this pathogen. These results do not deserve consideration because Mitra worked under the assumption that like other wheat bunt pathogens, *H. indica* causes seedling infection, which has been disproved by later workers. The possibility of infection in the treated plots by air-borne sporidia from distant fields or from the soil borne teliospores already present there cannot be ruled out. Moreover Mitra did not show the effect of these treatments on spore germination.

It has been shown in the preceding pages that sporidia reach the developing grain through the glumes. The possibility of protecting the plant by fungicidal spray has been explored. Of the four fungicides selected for the purpose when tested *in vitro*, all are effective at 2000 ppm concentration but at
lower concentrations of 400 ppm and below, in two fungicides P461 and Aureofungin the sporidial germination is partially inhibited. However, when sprayed on plants, P461 and Aureofungin gave complete control of the disease at both 2000 and 500 ppm concentrations, while the other two do not. This may be due to the production of some metabolites through interaction between fungicide and host which prevented the establishment of infection.

The ideal way of controlling plant diseases is by the use of resistant varieties. It has been shown that for getting good infection with *N. indica*, the isolates must be in fine sporulating condition. During the present investigation, a new technique described under "Material and Methods" has been devised, which amply satisfies the objective. Reaction of ninety wheat varieties has been studied against a mixture of isolates of *N. indica*. Of these eleven proved to be resistant. Unfortunately all the commercial wheat varieties in India prove to be susceptible but the redeeming feature is the locating of resistant varieties which can be utilised in the future breeding programme. Resistance is more in *Durum* and *Triticales* than *Aestivums*. The results are in agreement with Bedi et al. (1949). Most of the commercial wheats in India are aestivums, which cross with difficulty with *durum* or *triticale* because of different chromosome numbers. One aestivum, Nainari 60 has been found to be resistant which lacks desirable agronomic characters. This can be profitably used in the future breeding programme after
testing with the individual races of the pathogen.

Free amino acid content of the ears of two wheat varieties has been determined by paper chromatography. One belongs to the resistant and the other to susceptible group. Both the varieties show the presence of same amino acids but the spots differ in the intensity of colour. Among the various amino acids alanine, glutamic acid, aspartic acid, arginine and asparagine are more prominent. Earlier in the nutritional studies, these amino acids individually supported good growth of *N. indica*. Alanine which is the best nitrogen source for the growth of the fungus appears to be more in NP 720 the susceptible variety, on the basis of colour development.

Studies on the physiologic specialization show the existence of seven races on the basis of host reaction. Although isolates 1 and 33 show the same differential reaction and are considered as same race, former is more virulent. In smut fungi, the study of physiologic specialization has been mostly based on a field collection of spores, which may be composed of more than one race. Stability of pathogenicity is, therefore, directly related to the genetic purity of the spore population. Some workers (Sampson and Western, 1938; Stakman and Christensen, 1927; Holton, 1953) have, therefore, suggested the use of mono or paired sporidial cultures. This difficulty is obviated in *N. indica* if one uses monosporidial cultures.

Occasionally serology has been used for differentiation
of physiologic races in fungal pathogens. Immunization of rabbits with homogenised mycelial suspension of two morphologically distinct isolates of the fungus demonstrated the antigenic property of the pathogen by the precipitation test. Beck (1934, 1938) working with some smut fungi found this method well suited for demonstrating antigenicity. Presence of antigen in the culture filtrate confirms the results obtained by Tempel (1958) and Hegde et al. (1968) indicating the possible use of it for immunization. It is also observed that with the increase in age of culture, more antigenic substance is secreted into the medium. Precipitin tests carried out with antisera of the isolates with mycelial extract or cultural filtrate of 26 isolates reveal that they contain similar antigenic material on the basis of which they can be separated into definite serological groups. These results compare favourably with those of pathogenic specialization.

For quick screening of varieties against a particular disease Fedotova (1938, 1939), Doubly et al. (1960), Dounine (1966) have employed serodiagnostic technique with considerable success. The same technique has also shown good results in the case of six varieties tried for the same purpose.