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## SEED MYCOFLORA OF GREEN GRAM

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### ABSTRACT

The per cent incidence of seed mycoflora and seed germination of two varieties of green gram or mungbean (*Vigna radiata* L. var. Pant-2 and Type-44) were studied in freshly collected, six months stored and one year stored seeds. In all, 25, 23 and 25 fungal species were isolated from the seeds of var. Pant-2 collected from godown, shopkeepers and crop field respectively and 22 fungal species were isolated from var. T 44 collected from godown using agar plate and blotter techniques. Seed mycoflora superficially associated with the freshly collected seeds of both the varieties predominantly consisted of fungi belonging to genera *Alternaria*, *Cladosporium* and *Curvularia*. Dominant fungal species of *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium* and *Curvularia* were found to be associated with stored seeds. *Aspergillus flavus*, *A. niger*, *A. fumigatus* and *A. luchuensis* were found to be dominant on one year stored seeds. Deuteromycetes ranked first in number followed by phycomyetes. The number of fungal species was found more by the blotter technique in comparison to the agar-plate technique. The fungal population increased with increasing storage periods.

**KEY WORDS :** Green gram, seed mycoflora, collection place, storage

Seeds carry a wide range of microorganisms either externally or internally and these organisms become active in favourable conditions and cause extensive damage to the seeds and severe diseases on crops raised from them. About 90 per cent of all food crops grown are propagated by seed and these crops are attacked by devastating seed-borne diseases (Neergaard, 1986). Losses due to storage fungi in an Indian condition may be as high as 30 per cent of the total harvest (Neergaard, 1977). The seed borne mycoflora which is responsible for the reduction of seed quality, varies from place to place according to local conditions (Neergaard, 1967). The moist grain provides a favourable medium for the fungi to grow until it is dried to safe moisture level of 12 per cent (Palaniswami *et al.*, 1989). Seed mycoflora, particularly of green gram, has been studied already by some workers. In view of the above facts, the seed mycoflora of green gram

(*Vigna radiata* L.) in relation to collection place, varietal difference and storage were studied.

### MATERIALS AND METHODS

Freshly harvested seeds of two varieties of green gram or mungbean (var. Pant-2 and Type-44) were procured from the crop fields, godown (Government Agricultural Trial and Demonstration Centre, Varanasi) and shopkeepers (Gyanpur market) at Bhadohi district of Uttar Pradesh. Seed lots were dried under direct sunlight till they attain safe moisture level of around 5 per cent. The seeds were then stored in earthenware pots covered with lids under laboratory conditions for one year. Samples were withdrawn after six months and one year of storage and seed infection and percentage of seed germination were recorded simultaneously. For studying seed mycoflora in relation to

collection place, only one variety (Pant- 2) was selected.

Standard health testing techniques recommended by International Seed Testing Association (ISTA, 1985) were followed for the above study. The isolation of external seed mycoflora was done by plating seeds directly on sterilized petri plates containing potato dextrose agar (PDA) medium in aseptic condition. The seeds were also plated in Petri plates on sterilized and moist three layered blotters. For the isolation of endophytic seed-borne fungi, the seeds were surface sterilized with sodium hypochlorite (0.2% available chlorine) for five minutes and washed with sterilized distilled water and were then plated on sterilized petri plates both on PDA and moist

three layered blotters. The same procedures were followed for isolating microfungi at all stages. One hundred seeds of each variety were subjected to isolation of seed-borne fungi and to calculate percentage of occurrence and seed germination of seed borne fungi.

Five seeds per Petri plate were plated using both the methods. The plates were then incubated at  $22 \pm 2^{\circ}\text{C}$  under alternating cycles of 12 h light (artificial day light with fluorescent tube) and 12 h darkness. The plates were examined after 3 to 8 days of incubation and the slow growing fungi were transferred to separate agar plates so as not to miss any of the fungal flora. The isolates were purified by single spore monophthal tip culture method. Percentage of seed germination was also recorded.

Table I. Per cent incidence of seed-borne mycoflora of P2 collected from godown

| Fungal species                      | Sampling stages   |    |         |    |                   |    |         |    |                 |    |         |    |
|-------------------------------------|-------------------|----|---------|----|-------------------|----|---------|----|-----------------|----|---------|----|
|                                     | Freshly collected |    |         |    | Six months stored |    |         |    | One year stored |    |         |    |
|                                     | Agar              |    | Blotter |    | Agar              |    | Blotter |    | Agar            |    | Blotter |    |
|                                     | UT                | T  | UT      | T  | UT                | T  | UT      | T  | UT              | T  | UT      | T  |
| Per cent seed germination           | 70                | 72 | 74      | 76 | 74                | 80 | 80      | 84 | 72              | 76 | 78      | 80 |
| <i>Choanephora cucurbitarum</i>     | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Rhizopus nigricans</i>           | 12                | 4  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Alternaria alternata</i>         | 8                 | 4  | 4       | -  | 12                | -  | 4       | -  | 20              | 8  | -       | -  |
| <i>A. humicola</i>                  | 4                 | 4  | -       | -  | -                 | -  | -       | -  | 8               | -  | -       | -  |
| <i>Aspergillus candidus</i>         | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>A. flavus</i>                    | 8                 | -  | 4       | 4  | 8                 | 4  | -       | -  | 16              | -  | 12      | 4  |
| <i>A. fumigatus</i>                 | 8                 | -  | 4       | -  | 4                 | -  | -       | -  | -               | -  | -       | -  |
| <i>A. luchuensis</i>                | 8                 | 4  | 4       | 4  | 20                | 12 | 8       | 8  | 36              | 12 | -       | -  |
| <i>A. niger</i>                     | 12                | 8  | 8       | 4  | 28                | 20 | 8       | 12 | 32              | 16 | 16      | 12 |
| <i>A. nidulans</i>                  | 4                 | 8  | 4       | 4  | 8                 | 4  | 12      | 4  | 12              | 8  | -       | -  |
| <i>A. sulphureus</i>                | 8                 | 4  | -       | -  | 4                 | 4  | -       | -  | -               | -  | -       | -  |
| <i>A. terreus</i>                   | 8                 | -  | 12      | 8  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Bipolaris spicifera</i>          | 4                 | -  | 4       | -  | 8                 | -  | 4       | -  | 12              | -  | 8       | -  |
| <i>Cladosporium cladosporioides</i> | 4                 | -  | 4       | -  | 12                | 4  | 8       | -  | 20              | 8  | 12      | -  |
| <i>C. herbarum</i>                  | 8                 | 8  | 8       | 4  | 48                | 12 | 12      | 4  | 62              | 16 | 32      | 8  |
| <i>Curvularia lunata</i>            | 4                 | 4  | -       | -  | 8                 | 4  | -       | -  | 24              | 8  | -       | -  |
| <i>Fusarium chlamydosporum</i>      | 8                 | 4  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Penicillium citrinum</i>         | 4                 | -  | -       | -  | 8                 | -  | 4       | -  | 20              | -  | 12      | 4  |
| <i>P. javanicum</i>                 | -                 | -  | -       | -  | 4                 | -  | -       | -  | 8               | -  | -       | -  |
| <i>P. rubrum</i>                    | 8                 | 4  | -       | -  | 12                | 8  | -       | -  | 16              | 8  | -       | -  |
| <i>Phoma sp.</i>                    | 8                 | -  | -       | -  | 8                 | -  | -       | -  | 16              | -  | -       | -  |
| <i>Trichoderma viride</i>           | 4                 | 4  | -       | -  | 8                 | -  | 4       | -  | 20              | 8  | 12      | -  |
| Black sterile mycelium              | 4                 | -  | -       | -  | 16                | -  | -       | -  | 20              | 20 | -       | -  |
| Brown sterile mycelium              | -                 | -  | -       | -  | 4                 | -  | -       | -  | 8               | -  | -       | -  |
| White sterile mycelium              | 4                 | -  | -       | -  | 8                 | -  | -       | -  | 8               | -  | -       | -  |
| Total isolated                      | 23                | 12 | 10      | 6  | 19                | 9  | 9       | 4  | 18              | 9  | 7       | 4  |

- = Not present ; UT = Untreated ; T = Treated with 0.2% sodium hypochlorite

## RESULTS AND DISCUSSION

In all, 25, 23 and 25 fungal species were isolated from the seeds of Pant-2 collected from godown, shopkeepers and crop field respectively and 22 fungal species were isolated from var. T 44 collected from godown. Statistically a significant variation was recorded in relation to sampling stages and methods. The different methods were significant at  $P=0.05$ , sampling stages were significant at  $P=0.05$ .

From the data presented (Tables 1-4), it is evident that the seed mycoflora superficially associated with the freshly collected seeds of both the varieties predominantly consisted of field fungi belonging to the genera *Alternaria*, *Cladosporium* and *Curvularia*. The species of *Aspergillus* and *Penicillium* were found in minimum frequency

during freshly collected seeds and maximum frequency during the storage condition. In storage, there are mostly the species of *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium* and *Curvularia*. Similar results were observed by Randhawa and Aulakha (1981). The members of fungi imperfecti were also very common and frequent. Some species of *Rhizopus* and *Chaetomium* were also detected.

The results emphasize that the climate of Varanasi and Bhadohi districts favoured the growth of *Aspergillus flavus*, *A.niger*, *A.funigatus* and *A.luchuensis* more dominantly in one year stored seeds of the test crops. *Aspergillus flavus* and *A.niger* were found to be the commonest species associated with the stored seeds of both the crops in this region. Co-dominant species were *Alternaria*

Table 2. Per cent incidence of seed-borne mycoflora of P2 collected from shop-keepers

| Fungal species                      | Sampling stages   |    |         |    |                   |    |         |    |                 |    |         |    |
|-------------------------------------|-------------------|----|---------|----|-------------------|----|---------|----|-----------------|----|---------|----|
|                                     | Freshly collected |    |         |    | Six months stored |    |         |    | One year stored |    |         |    |
|                                     | Agar              |    | Blotter |    | Agar              |    | Blotter |    | Agar            |    | Blotter |    |
| Per cent seed germination           | UT                | T  | UT      | T  | UT                | T  | UT      | T  | UT              | T  | UT      | T  |
| <i>Rhizopus nigricans</i>           | 72                | 74 | 73      | 76 | 75                | 82 | 80      | 85 | 60              | 65 | 75      | 77 |
| <i>Chaetomium</i> sp.               | 4                 | -  | 4       | -  | 4                 | -  | -       | -  | -               | -  | 4       | -  |
| <i>Alternaria alternata</i>         | 8                 | 4  | 8       | 4  | 16                | -  | 8       | 4  | -               | -  | -       | -  |
| <i>A.humicola</i>                   | 4                 | -  | -       | -  | -                 | -  | 4       | 8  | -               | -  | -       | -  |
| <i>Aspergillus candidus</i>         | 8                 | 4  | 4       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>A.flavus</i>                     | 8                 | -  | -       | -  | 8                 | 4  | 20      | -  | 32              | 8  | -       | -  |
| <i>A.luchuensis</i>                 | 28                | 12 | 12      | 8  | 24                | 20 | 16      | 8  | 48              | 16 | 40      | 20 |
| <i>A.niger</i>                      | 20                | 16 | 24      | 12 | 28                | 8  | 16      | 12 | 48              | 24 | 12      | 12 |
| <i>A.sulphureus</i>                 | 8                 | -  | -       | -  | 4                 | -  | -       | -  | -               | -  | 4       | -  |
| <i>A.terreus</i>                    | 12                | 8  | 4       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Bipolaris tetramera</i>          | 8                 | -  | -       | -  | 4                 | -  | -       | -  | 8               | -  | -       | -  |
| <i>Cladosporium cladosporioides</i> | 4                 | -  | 4       | 4  | 12                | 8  | -       | -  | 20              | 8  | 12      | 8  |
| <i>C.herbarum</i>                   | 12                | -  | 4       | 4  | 32                | 16 | 8       | 8  | 40              | 12 | 16      | 8  |
| <i>Curvularia lunata</i>            | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Penicillium chrysogenum</i>      | 4                 | -  | -       | -  | 4                 | -  | -       | -  | 16              | -  | -       | -  |
| <i>P.citrinum</i>                   | 12                | 4  | 4       | -  | 12                | 8  | -       | -  | 16              | 4  | 8       | 4  |
| <i>P.javanicum</i>                  | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>P.rubrum</i>                     | 4                 | 4  | 8       | -  | 8                 | -  | 4       | -  | 16              | -  | -       | -  |
| <i>Phoma</i> sp.                    | -                 | -  | -       | -  | 4                 | -  | -       | -  | 8               | -  | -       | -  |
| <i>Trichoderma</i> sp.              | 12                | 4  | -       | -  | 16                | -  | 8       | -  | 20              | -  | 4       | -  |
| Black sterile mycelium              | 8                 | -  | -       | -  | 4                 | -  | 8       | -  | -               | -  | -       | -  |
| Brown sterile mycelium              | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| White sterile mycelium              | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| Total no. of species isolated       | 22                | 8  | 11      | 6  | 16                | 6  | 9       | 5  | 12              | 6  | 8       | 5  |

- = Not present ; UT = Untreated ; T = Treated with 0.2% sodium hypochlorite



Table 3. Per cent incidence of seed-borne mycoflora of P2 collected from crop field

| Fungal species                      | Sampling stages   |    |         |    |                   |    |         |    |                 |    |         |    |
|-------------------------------------|-------------------|----|---------|----|-------------------|----|---------|----|-----------------|----|---------|----|
|                                     | Freshly collected |    |         |    | Six months stored |    |         |    | One year stored |    |         |    |
|                                     | Agar              |    | Blotter |    | Agar              |    | Blotter |    | Agar            |    | Blotter |    |
|                                     | UT                | T  | UT      | T  | UT                | T  | UT      | T  | UT              | T  | UT      | T  |
| Per cent seed germination           | 70                | 65 | 75      | 80 | 76                | 80 | 80      | 85 | 60              | 65 | 70      | 75 |
| <i>Azizopus nigricans</i>           | 12                | 13 | 8       | -  | 12                | 8  | 8       | 4  | 32              | 24 | 24      | 16 |
| <i>Collyria</i>                     | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | 8       | -  |
| <i>Chaetomium</i> sp.               | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Alternaria alternata</i>         | 12                | 8  | 8       | 4  | 20                | 4  | 12      | 8  | 32              | 24 | 20      | 12 |
| <i>A. humicola</i>                  | 8                 | -  | -       | 4  | 12                | 8  | -       | -  | 16              | -  | -       | -  |
| <i>Aspergillus candidus</i>         | 4                 | -  | -       | -  | 8                 | -  | -       | -  | 12              | -  | -       | -  |
| <i>A. flavus</i>                    | 16                | 8  | 12      | 4  | 32                | 12 | 12      | 12 | 28              | 20 | 16      | 12 |
| <i>A. fumigatus</i>                 | 8                 | -  | -       | -  | -                 | -  | -       | -  | 24              | 12 | 16      | 8  |
| <i>A. luchuensis</i>                | 8                 | 4  | 4       | 4  | 8                 | -  | -       | -  | 28              | 12 | 16      | 8  |
| <i>A. niger</i>                     | 20                | 8  | 12      | 8  | 36                | 24 | 16      | 12 | 76              | 44 | 56      | 36 |
| <i>A. sulphureus</i>                | 4                 | -  | -       | -  | 8                 | -  | -       | -  | 16              | -  | -       | -  |
| <i>A. terreus</i>                   | 8                 | 4  | 4       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Aspolaris tetramera</i>          | 4                 | -  | -       | -  | 4                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Cladosporium cladosporioides</i> | 4                 | 4  | -       | -  | 4                 | 4  | -       | -  | -               | -  | -       | -  |
| <i>C. herbarum</i>                  | 16                | 12 | 8       | 4  | 32                | 8  | 12      | -  | 40              | 12 | -       | -  |
| <i>Curvularia clavata</i>           | 12                | -  | 4       | -  | 16                | 8  | 4       | -  | 20              | 4  | 4       | -  |
| <i>C. pallescens</i>                | 8                 | 12 | 8       | 4  | 20                | 12 | 8       | 4  | 24              | 8  | -       | -  |
| <i>Penicillium chrysogenum</i>      | 8                 | -  | -       | -  | 12                | 4  | -       | 4  | -               | -  | -       | -  |
| <i>P. citrinum</i>                  | 12                | 4  | -       | -  | 24                | 16 | 12      | -  | 36              | 8  | -       | -  |
| <i>P. javanicum</i>                 | 4                 | 8  | 4       | 4  | -                 | -  | -       | -  | 16              | -  | -       | -  |
| <i>P. lanosum</i>                   | 8                 | 4  | -       | -  | 12                | 8  | 4       | -  | 16              | 8  | -       | -  |
| <i>Phoma</i> sp.                    | -                 | -  | -       | -  | 8                 | 4  | -       | -  | 16              | -  | -       | -  |
| <i>Trichoderma harzianum</i>        | 16                | 4  | 8       | 4  | 16                | 8  | 4       | 4  | 24              | -  | 12      | 4  |
| Black sterile mycelium              | 8                 | -  | -       | -  | 4                 | -  | -       | -  | -               | -  | -       | -  |
| White sterile mycelium              | 4                 | -  | -       | 4  | -                 | -  | -       | -  | -               | -  | -       | -  |
| Total no. of species isolated       | 24                | 13 | 11      | 10 | 19                | 14 | 10      | 7  | 17              | 11 | 9       | 7  |

- = Not present ; UT = Untreated ; T = Treated with 0.2% sodium hypochlorite

*alternata*, *Aspergillus candidus*, *Cladosporium* and *Trichoderma*. Similar results were observed by Dwivedi (1992).

The per cent seed germination were found to be increased during six months and decreased during freshly collected seeds and one year stored seeds. Decreased per cent seed germination during freshly collected seeds might be due to the hard seed coat. The decrease in per cent seed germination in one year stored seeds might be due to the presence of more microorganisms than those of six months stored seeds. The result emphasized that the proper seed germination required the definite time duration of storage not prolonged with six months. The association of the moulds with seeds is of great significance not only from the view point of the

decrease in quality especially food value but loss of germination power of the seeds also. The presence of *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *C. herbarum* and *Trichoderma* sp. in such a high percentage may be the reason for low germination of test seeds. These fungi are known to cause serious loss and seedling infestation in various crops. Variation in the number and species of external and internal seed mycoflora was observed among both the varieties. Differences in the percentage of different fungal colonies developing from different varieties were also observed. The physico-chemical nature of the seed and agricultural operations might have influenced the distribution of mycoflora as both the varieties were collected from the same place and at the same time.

Table 4. Per cent incidence of seed-borne mycoflora of T44 collected from godown

| Fungal species                      | Sampling stages   |    |         |    |                   |    |         |    |                 |    |         |    |
|-------------------------------------|-------------------|----|---------|----|-------------------|----|---------|----|-----------------|----|---------|----|
|                                     | Freshly collected |    |         |    | Six months stored |    |         |    | One year stored |    |         |    |
|                                     | Agar              |    | Blotter |    | Agar              |    | Blotter |    | Agar            |    | Blotter |    |
|                                     | UT                | T  | UT      | T  | UT                | T  | UT      | T  | UT              | T  | UT      | T  |
| Per cent seed germination           | 74                | 75 | 76      | 78 | 76                | 78 | 78      | 80 | 60              | 65 | 68      | 70 |
| <i>Chaenophora cucurbitarum</i>     | 8                 | 4  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Rhizopus nigricans</i>           | 8                 | 4  | -       | -  | 4                 | -  | -       | -  | 16              | -  | -       | -  |
| <i>R.oryzae</i>                     | 4                 | -  | -       | -  | -                 | -  | -       | -  | 8               | -  | -       | -  |
| <i>Chaetomium</i> sp.               | 8                 | -  | -       | -  | -                 | -  | -       | -  | 8               | -  | -       | 8  |
| <i>Alternaria alternata</i>         | 4                 | -  | -       | -  | 20                | 12 | -       | -  | 48              | 28 | 16      | 8  |
| <i>A.humicola</i>                   | 4                 | -  | -       | -  | 4                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Aspergillus flavus</i>           | 8                 | 4  | -       | -  | 12                | 4  | 4       | -  | 48              | 20 | -       | -  |
| <i>A.fumigatus</i>                  | 8                 | -  | 4       | -  | 4                 | -  | -       | -  | -               | -  | -       | -  |
| <i>A.luchuensis</i>                 | 4                 | -  | -       | -  | 12                | 4  | -       | -  | -               | -  | -       | -  |
| <i>A.niger</i>                      | 28                | 12 | 8       | 4  | 48                | 28 | 16      | 8  | 60              | 48 | 40      | 36 |
| <i>A.sulphureus</i>                 | 4                 | -  | -       | -  | -                 | -  | -       | -  | 4               | -  | -       | -  |
| <i>A.sydowi</i>                     | 8                 | -  | 4       | -  | 12                | -  | -       | -  | 16              | 4  | -       | -  |
| <i>A.terreus</i>                    | 4                 | -  | 12      | 4  | -                 | -  | -       | -  | 4               | -  | -       | -  |
| <i>Cladosporium cladosporioides</i> | 8                 | 4  | -       | 4  | 36                | 12 | 20      | 28 | 40              | 16 | 20      | 12 |
| <i>C.herbarum</i>                   | 6                 | 4  | 4       | 4  | 28                | 16 | 8       | 8  | 36              | 8  | 8       | -  |
| <i>Curvularia lunata</i>            | 4                 | -  | -       | -  | -                 | -  | -       | -  | -               | -  | -       | -  |
| <i>Penicillium</i> sp.              | 12                | 4  | 8       | 4  | 28                | 12 | 20      | 28 | 44              | 16 | 28      | 16 |
| <i>P.javanicum</i>                  | 8                 | -  | 8       | 4  | 8                 | -  | -       | -  | -               | -  | -       | -  |
| <i>P.lanosum</i>                    | 8                 | 4  | 16      | 8  | 16                | -  | 20      | 4  | 24              | -  | -       | -  |
| <i>Phoma</i> sp.                    | -                 | -  | -       | -  | 4                 | -  | -       | -  | 16              | -  | -       | -  |
| Black sterile mycelium              | 8                 | -  | -       | -  | 4                 | -  | -       | -  | -               | -  | 8       | -  |
| Brown sterile mycelium              | 4                 | -  | -       | -  | -                 | -  | -       | -  | 20              | 8  | -       | -  |
| Total isolated                      | 21                | 8  | 8       | 7  | 15                | 7  | 6       | 5  | 15              | 8  | 6       | 5  |

- = Not present ; UT = Untreated ; T = Treated with 0.2% sodium hypochlorite

The number of fungal species was found more by the blotter technique in comparison to the agar plate technique. This may be attributed to the reason that some slow growing fungi could not grow successfully in culture plates in comparison with fast growing fungi. The other reason might be the selective nature of the culture medium which might not have favoured the growth of some other fungi. The blotter method is thus proved to be better for the study of seed mycoflora in comparison to the agar plate method.

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