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# Seed Quality and Health Status of (*Vigna unguiculata* L. Walp) Farmer's Saved cowpea Seeds Collected from Different Locations of Western Odisha

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**ABSTRACT: A study was conducted in the Department of Seed Science and Technology and Department of Plant Pathology, College of Agriculture, OUAT, Bhawanipatna to determine the seed health status of cowpea seeds with respect to presence of seed borne myco-flora associated with cowpea seeds. Particularly in treated seeds and their impact on various seed quality parameters. A total of ten cowpea seed samples were taken from farmers of various districts throughout Western Odisha. From both the methods i.e., blotter paper and the PDA, several plant pathogens were detected such as *Aspergillus niger, Aspergillus flavus, Fusarium equiseti, Rhizopus* sp., *Macrophomina phaseolina, Pseudomonas* sp., and *Xanthomonas* sp. In several seed lots, microbial infection was ranged from (2.07-4.85%) using blotter paper methods. In the blotter paper method, infection by *Aspergillus niger* (4.85%) was highest, followed by *Aspergillus flavus* (4.47%), *Pseudomonas* sp. (4.37 %), *Fusarium equiseti* (3.55%), Rhizopus sp. (2.52%), and *Xanthomonas* sp. (2.07 %). In the PDA and Blotter paper methods, seed mycoflora identification varied from 5.75–11.25 % and 12.75–30.25 %, respectively. Blotter paper methods shows a higher number of seed mycoflora than PDA methods as the seeds are not treated by (0.1%) $HgCl_2$.**

**Keywords:** Seed quality, mycoflora, seed health, farmer's saved seed, cowpea.

## INTRODUCTION

Cowpea is an annual herbaceous plant in the fabaceae family. Cowpea seeds are an excellent source of protein (22.24%), calories (336kcal), minerals, and vitamins (Gondwe *et al.,* 2019). Cowpea is known as "poor man's meat" because of the high protein content in the grains and leaves. As a result, it is vital to the livelihoods of relatively impoverished people in developing countries in the tropics, especially where animal protein is scarce. Cowpea can be considered as a multifunctional crop because it provides food for people, cattle feed, concentrate for farm animal, fixes atmospheric nitrogen, hay, silage, and green manure, along with many other things.

Despite of the nutritional qualities the cowpea seeds are very much prone to the attack by seed borne mycoflora. The presence of these seed mycoflora has a long-term deleterious effect on seed health and yield. Fungi, which are the most prevalent pathogens, have a substantial influence in seed quality degradation. When these potential plant pathogenic fungi were found in large numbers on cowpea, it raises the possibility of a threat to crop productivity. To ensure effective seed germination and subsequent crop emergence, there must be no mycoflora on the seed. The association between seed quality parameters and seed mycoflora is intricate, and it varies depending on how farmers kept cowpea seed for planting (Njonjo *et al.,* 2019). Seed germination is an excellent sign of how healthy a seed seems, but the low germination rate was caused by microorganisms that impaired the development of new and emerging shoots.

In India, farmers save even more than 70% of seeds, whereas only 30% of quality (certified) seeds are accessible. To increase cowpea seed production and productivity, farmers must procure quality certified seeds at the right time, and if they are using their own

farm saved seeds, they must be aware of proper storage conditions to enhance the performance of their low vigour seeds before sowing in the main field. As a result, the quality of seeds stored by farmers is essential. Farmers' saved cowpea seeds from the western zones of Odisha were evaluated seed quality and mycoflora associations. To utilise high-quality seeds for better establishment of crop.

## MATERIALS AND METHODS

The current study investigates on the seed quality and mycoflora association in farmer-saved cowpea seeds from various districts in Western Odisha. The research was conducted in the Department of Seed Science and Technology and Department of Plant Pathology, College of Agriculture, Bhawanipatna, OUAT during 2020-21 academic year. The design of experiment was CRD with four replications.

## METHODOLOGY

Each sample was physically inspected with the aid of a magnifying lens linked to the physical purity board and then divided into pure seeds, other seeds (such as crop seeds and weed seeds) and inert material. The weight of the physical pure seed was calculated, and the findings were reported in percentages up to one decimal place using the formulas below.

Physical pure seed (%) = Weight of pure seed (g)/ Total sample weight (g) × 100

Ten seeds were randomly selected from each replication and measured with callipers (mm) to determine seed length and width. Random of 100 seeds were drawn from each sample and counted manually and their weight was recorded in grams with the help of digital electronic balance. The moisture content of the seeds was determined using the hot air oven method which involved crushing the 10 g seeds in a grinding mill and drying them at 103±2 °C for 2 hours in a hot air oven according to ISTA regulations. On a weight basis, the percentage of moisture content was determined.

Moisture content (%) = (W2-W3/W2-W1) × 100

Where, W1 = Weight of empty container with its cover (g)

W2 = Weight of container with its cover and seeds before drying (g)

W3 = Weight of container with its cover and seeds after drying (g)

ISTA Rule was used to determine the germination percentage (Anonymous, 1996). In four replications, 100 seeds from each sample / treatment were maintained for germination using the between paper method. Seeds were counted at random and placed in rows on germination paper with uniform spacing. For eight days, seed-filled rolled paper towels were stacked vertically in a seed germinator cabinet at a constant temperature of 25±2°C and 90±2% relative humidity. The seedlings were categorized into normal, abnormal, freshly ungerminated seeds and dead seeds. As a result, the germination % was calculated using normal seedlings. The germination rate was estimated using the formulas below in percentages up to whole numbers.

Germination (%) = Number of normal seedlings/Total number of seed placed for germination × 100

Randomly ten seedlings were taken from each replication to measure the shoot length. The distance between the collar and the tip of the shoot was measured in centimetres, and a mean value was calculated. The same ten seedlings that were used to measure shoot length were also utilised to measure root length. The distance between the collar area and the primary root tip was measured in centimetres. After then, the average value was computed. Ten normal seedlings were wrapped in butter paper and dried in a hot air oven at 75±2°C for 6 hours. The seedlings were weighed and expressed in grams after cooling for 30 minutes in a desiccator. Ten normal seedlings from each replication were selected for calculation of vigour index (Abdul-Baki & Anderson 1973) by adopting the formula given below.

Seedling vigour index I = Germination % x Seedling length (cm)

Seedling Vigour index II was calculated by adopting the formula as given below-

Seedling Vigour index II = Germination % x Seedling dry weight (g)

Speed of germination was monitored on a daily basis (radical emergence). The following formula was used to calculate germination speed (Maguire, 1962).

Germination speed = n1/d1+n2/d2+n3/d3 + ----------

Where 'n' denotes the number of germinated seeds and 'd' denotes the number of days.

Tetrazolium test was performed as per Moore, 1985. To activate dehydrogenase enzymes, three replications of 50 seeds were immersed in 50ml water for 16 hours at 25°C. The seeds were then cut longitudinally to divide the embryo into two equal halves, and then stained in 0.5 % tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride) in petri plates for 4 hours at 38°C. The solvent was then drained out, and the seeds were washed in water before being inspected under magnification. The number of seeds that were completely red when stained were considered viable seeds and were represented as a percentage.

**Standard blotter method.** Three pieces of 200 mm blotting paper were soaked with distilled water and placed on 200 mm sterilised Petriplates after draining excess water. 100 seeds per Petriplate were equally spread across three layers of fully wet sterilised blotters using forceps soaked in 70% alcohol and heated over a flame. Petriplates were then incubated for seven days at 28±2°C with a 12/12 hour alternating light and dark phase. The plates were checked on a regular basis during the incubation period. After seven days of incubation, the fungal growth was examined under a stereoscopic binocular microscope. The plates were removed, and observations were made on the type of fungus and the amount of fungi present.

**Agar Method.** 100 seeds were placed at equal distance in Petriplates containing 200 × 30 ml of PDA that had previously been filled with PDA using forceps soaked in 70% alcohol and heated over a flame. The flaming was repeated after each plate had been filled. The Petriplates were incubated for seven days as directed in

the usual blotter method. After seven days of incubation, the fungal growth was examined under a stereoscopic binocular microscope. Plates were taken out and observations on the kind of fungus and the amount of fungi were recorded, same as the blotter method. Both operations were carried out in aseptic environments with laminar air flow to prevent contamination. In both methods, 100 seeds were placed in each plate, evenly spaced from one another, and 10 plates were utilised for each treatment, yielding 4000 seeds in total.

## RESULTS AND DISCUSSION

### Evaluation of seed quality parameters

**Physical pure seed percentage.** The physical pure seed percentage of all the farmers' collected seed varied from 90.5 % (Rairakhol, Sambalpur) to 97.1 % (Bhawanipatna, Kalahandi) as shown in Table 1, which was below than the Indian Minimum Seed Certification Standards (*i.e.,* 98 %) for cowpea seed. The proportion of inert matter in all collected seeds from different places ranged from 2.9 % (Bhawanipatna, Kalahandi) to 9.5 % (Rairakhol, Sambalpur). This may be attributed by poor harvesting, threshing and cleaning of seeds and leaded to poor seed quality. The contaminants occurred in maximum range for the other crop seeds, weed seeds and inert matters, all of which exceeded the minimal seed certification limits, indicating that the farmer-saved cowpea was of poor quality. Naik (2015) in soybean, Rahman *et al.* (2017) in okra seeds, and Kamara *et al.,* (2019) in cowpea seed all revealed differences in physical purity for various farmers saved seeds.

**Germination %.** Seed samples from different parts of western Odisha showed significant variances in normal seedling as shown in Table 2. Germination rates ranged from 59 % to 84 %, depending on region and seed source. The minimum gratitude of germination percentage in seed sample (59%) was achieved by collected seeds from Khariar, Nuapada agro ecological zones, and was similar to Kantamal, Boudh collection seed (54%) which had a lower germination percentage in comparison to the minimum seed certification standards (75%) for cowpea seed. It might be owing to the fact that rainfall, humidity, and temperature all vary significantly throughout agro-ecological zones. The variability might also be linked to the genetic composition of each seed collected, as well as environmental factors like as production, harvesting, packing, and storage.

Germination is a biological process that is controlled by a variety of variables, one of which is genetic behaviour. In okra seeds, Sarkar *et al.* (2015); Rahman *et al*. (2017) found a wide range of variances in standard germination %, as found by Njonjo *et al.* (2019) in cowpea. Farmers frequently retain and recycle seeds for several seasons, resulting in the development of seed mycoflora, which had a detrimental influence on seeds, generating more abnormal seedlings and post-emergency mortality in their own saved seeds.

**Speed of germination.** Before complete germination, the speed of germination was to count preliminary germination at a standard period given for cowpea seeds. Farmers who saved seed from Bhawanipatna (21.84) with the fastest speed of germination value, SVI-I and SVI-II, had the highest germination percentage (Table 2). These findings corroborate with previous study in cowpea by Kamara *et al.,* (2019); Spears *et al.,* (2002), who found that seeds with high vigour can tolerate stress during germination and germinate faster than seeds with low vigour.

**Root length and shoot length (cm).** The root length of cowpea seeds sampled showed a lot of variation (Table 2). Root length is one of the good markers for seed vigour assessments, and it can help seedlings establish more quickly. Iqbal (2015) observed similar findings in cowpea, stating that seedling growth and development displays good seed production as vigorous seedling establishment leads to realising maximum potential of crops. The findings of shoot length for the divergence genotype were remarkable, and it serves as a key measure for estimating seedling vigour, which may also aid in seedling growth and development.

The length of the shoots ranged from 2.29 cm to 3.33 cm. The genetic composition of the variations/genotypes may account for the difference in seedling height among all the gathered seeds. The current findings are in accordance with those of Gan *et al.* (2003) previous study. According to Kamara *et al.* (2019), cowpea localized varieties or genotypes from diverse locations had the shortest root and shoot lengths compared to improved varieties. This might be one of the reasons for the poor seedling vigour. Also, differences in shoot length across farmers' seeds might be attributable to differences in genotype genetic makeup.

**Seedling dry weight (g).** The overall mean for seedling dry weight was 1.42g while range of seedling dry weight varied from 1.15g to 1.86g (Table 2). Variation in dry weight for different local seed collections from various places might be due to difference in genetic constitution of various genotypes

**Seed vigour index I and II.** The germination % and seedling (root & shoot) length were the most important criteria in analysing the SVI-I, which demonstrated substantial variation across all seed collections, perhaps due to seed genetic composition (Table 2). The seedling vigour index-II is a crucial means of determining seedling survival and development following germination. Seed samples from different agro-ecological sites had significantly different seedling vigour indexes, which is consistent with findings by Bishaw *et al*. (2012), who observed similar findings when working on wheat from several places in Ethiopia, and Njonjo *et al.* (2019) in cowpea seed used by farmers in Makueni and TaitaTaveta countries in Kenya.

**Seed moisture (%).** All of the farmers' seed samples had a moisture level higher than the minimum seed certification standard for cowpea (9.0%) as shown in Table 1. Due to differences in seed drying and storage packing, individual farmers' seeds had variable moisture levels. Cowpea seeds were more sensitive to storage fungus and storage grain insects because they were

stored with higher moisture content at the farmer level, which lowered germination percentage and increased abnormal and dead seedlings in this study. Similar results were observed by Tonu *et al.* (2017) in wheat and Rahman (2017) in okra seed. As a result, before storing seeds in an airtight container, they needed to be dried to their optimum moisture level.

**100 seed weight (g).** There was a substantial variance in 100 seed weight across farmer seed samples taken from different districts (Table 1). Seeds of same size or weight were shown to be superior in terms of germination, speed and seedling vigour index. Abduhu (2007) found similar results with okra seeds, finding that test weight varied depending on location, seed source, and variety. The variances in seed weight in this cowpea seed sample are owing to the presence of broken or damaged seeds, which might be a contributing factor in reduced germination. Some of the seeds sampled had a maximum 100 seed weight of 19.7 g and a minimum 100 seed weight of 9.9 g. This might be owing to the fact that different kinds/genotypes were collected from various agro-ecological regions. However, Mandanzi *et al.* (2010) evaluated the impact of seed size and planting strategy on soybean seed stand establishment and discovered that large seeds have an advantage for emerging when placed at the sown level, but tiny and medium seeds cannot emerge when sown at the field level.

**Identification of seed mycoflora.** Microbes such as *Macrophomina phaseolina, Aspergillus flavus, Fusarium equiseti, and Alternaria* sp. were found in this PDA method at 3.10, 1.58, 1.13, and 2.0 %, respectively (Table 3). Although their infection rate is 7.8%, it reaches a level of 11.25 % in the seed lot. These reports corroborated our findings from Kumar and Singh (2001) ; Hirwani (2001). (2016). Blotter paper was also used to record the mycoflora status of cowpea seeds. Individual microorganism evaluations were investigated. Different bacteria were found, including *Aspergillus niger, Aspergillus flavus, Fusarium equiseti, Rhizopus* sp., *Pseudomonas* sp., *and Xanthomonas* sp., Pradhan (2019) both observed similar findings (2021). An average of 2.075 - 4.85 % of microbes were found in the seed lot, with *Aspergillus niger* (4.85 % ), *Aspergillus flavus* (4.475 % ), *Pseudomonas* sp. (4.375 % ), *Fusarium equiseti* (3.55 % ), *Rhizopus* sp. (2.525 % ), and *Xanthomonas* sp. (2.075 %). Kumar and Singh (2001) observed similar results when dealing with pigeon pea seed samples.

This blotter paper technique was used to record infection percentages ranging from 30.25 to 12.75 % in the current investigation (Table 4). Swarna from the Bhawanipatna collection had the lowest infection, while Ankur Gomati from Kantamal, Boudh had the highest seed infection, at 30.25 % in this Blotter paper method, but it ranged from 9.25 to 20.75 % in both varieties in this PDA method, as reported by Sadhu (2014); Naik (2015), and Hirwani (2015; 2016). The findings of previous researchers were presented in light of the current discoveries.

These earlier workers' reported coroborates with the current findings. As a result, *Macrophomina phaseolina* and *Alternaria* sp. are internal seed borne, according to the current findings. These findings matched those of Kumar (2016); Pradhan (2016), who had initially reported on our present work. Earlier researchers had gotten similar results. Our current findings are backed up by these discoveries. Genotypes of Rairakhol, Sambalpur had the lowest seed germination (54%) owing to the greatest seed moisture *percentage* (11.3%) and the highest seed mycoflora Association (20.75%). Similarly, Khariar, Nuapada collected genotypes had the greatest germination rate of 84.0 % due to the lowest seed moisture (9.3%) and seed mycoflora (9.35%). Thus, the current findings acquired adequate support from the experiment that examining the relationship of seed mycoflora directly impacting seed quality measures is essential, which was also obtained from the findings of Patil *et al.* (2012), Rathod *et al.* (2012); Biemond *et al.* (2013). These findings matched those from our recent findings.

**Table 1: Physical purity analysis, 100 seed weight, seed moisture (%) and viability % of cowpea seed collected from various places of Western Odisha.**

| Sr. No. | Place of Collection | Physical purity % | 100 seed weight (g) | Seed moisture (%) | Viability % |
|---|---|---|---|---|---|
| 1. | Rairakhol, Sambalpur | 90.5 | 16.6 | 12.2 | 82.6 |
| 2. | Kantamal, Boudh | 92.4 | 11.7 | 11.3 | 86.2 |
| 3. | Tarang, Deogarh | 96.7 | 16.1 | 9.8 | 90.8 |
| 4. | Jujomura, Sambalpur | 95.6 | 18.8 | 9.6 | 90.2 |
| 5. | Dunguripali, Sonepur | 91.7 | 15.3 | 12.3 | 92.4 |
| 6. | Salebhata, Balangir | 94.2 | 15.8 | 9.8 | 93.2 |
| 7. | Attabira, Bargarh | 90.6 | 10.8 | 9.1 | 88.6 |
| 8. | Rupra road, Kalahandi | 91.2 | 9.9 | 9.2 | 84.8 |
| 9. | Bhawanipatna, Kalahandi | 97.1 | 19.7 | 9.3 | 92.6 |
| 10. | Khariar, Nuapada | 93.5 | 17.1 | 11 | 84 |
| Mean | | 93.35 | 15.18 | 10.36 | 88.54 |
| SEm ( ± ) | | 1.48 | 0.04 | 0.21 | 0.32 |
| CD (0.05) | | 4.29 | 0.12 | 0.62 | 0.94 |
| CV (%) | | 3.17 | 0.54 | 4.09 | 0.73 |

**Table 2: Status of seed germination and vigour index of cowpea collected from various places of Western Odisha.**

| Sr. No. | Place of Collection | First count | Normal seedling (%) | Speed of germination | Shoot length (cm) | Root length (cm) | Dry weight (g) | SVI-I | SVI-II |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Rairakhol, Sambalpur | 43 | 59 | 17.10 | 2.29 | 2.11 | 1.17 | 259.6 | 69.03 |
| 2 | Kantamal, Boudh | 31 | 54 | 14.84 | 2.45 | 3.17 | 1.29 | 370.92 | 85.14 |
| 3 | Tarang, Deogarh | 46 | 79 | 20.10 | 3.05 | 2.66 | 1.49 | 462.51 | 129.69 |
| 4 | Jujomura, Sambalpur | 56 | 76 | 19.42 | 2.77 | 2.62 | 1.81 | 409.64 | 137.56 |
| 5 | Dunguripali, Sonepur | 34 | 80 | 19.10 | 3.13 | 3.02 | 1.31 | 492 | 104.8 |
| 6 | Salebhata, Balangir | 31 | 81 | 20.15 | 3.33 | 3.11 | 1.64 | 508.76 | 120.69 |
| 7 | Attabira, Bargarh | 28 | 77 | 16.93 | 2.85 | 2.68 | 1.21 | 425.81 | 93.17 |
| 8 | Rupra road, Kalahandi | 42 | 60 | 15.66 | 2.56 | 2.18 | 1.15 | 284.4 | 69 |
| 9 | Bhawanipatna, Kalahandi | 51 | 84 | 21.84 | 3.26 | 2.88 | 1.86 | 515.76 | 156.24 |
| 10 | Khariar, Nuapada | 26 | 52 | 11.37 | 2.39 | 2.26 | 1.25 | 241.8 | 65 |
| Mean | | 38.80 | 70.20 | 17.65 | 2.81 | 2.67 | 1.42 | 397.12 | 103.02 |
| SEm ( ± ) | | 1.00 | 2.08 | 0.36 | 0.06 | 0.05 | 0.03 | 3.80 | 2.70 |
| CD (0.05) | | 2.91 | 6.03 | 1.03 | 0.16 | 0.15 | 0.08 | 11.02 | 7.84 |
| CV (%) | | 5.16 | 6.30 | 4.03 | 3.94 | 3.89 | 3.95 | 1.91 | 5.24 |

**Table 3: Seed mycoflora status of cowpea seed by PDA (Treated with Mercuric chloride-$HgCl_2$ 0.1%).**

| Sr. No. | Place of Collection | *Macrophomina phaseolina* | *Aspergilusflavus* | *Fusarium equiseti* | *Aternaria sp.* | Infected seed % |
|---|---|---|---|---|---|---|
| 1. | Rairakhol, Sambalpur | 4.25 | 1.50 | 1.25 | 2.25 | **9.25** |
| 2. | Kantamal, Boudh | 4.25 | 2.50 | 1.25 | 3.25 | **11.25** |
| 3. | Tarang, Deogarh | 2.25 | 2.00 | 0 | 1.75 | **6.00** |
| 4. | Jujomura, Sambalpur | 3.25 | 1.75 | 1.50 | 1.50 | **8.00** |
| 5. | Dunguripali, Sonepur | 2.25 | 2.50 | 1.75 | 0 | **6.5** |
| 6. | Salebhata, Balangir | 3.00 | 0 | 1.00 | 2.25 | **6.25** |
| 7. | Attabira, Bargarh | 1.25 | 1.75 | 1.50 | 2.50 | **7.00** |
| 8. | Rupra road, Kalahandi | 3.25 | 2.50 | 0 | 2.50 | **8.25** |
| 9. | Bhawanipatna, Kalahandi | 2.75 | 0 | 1.25 | 1.75 | **5.75** |
| 10. | Khariar, Nuapada | 4.50 | 1.25 | 1.75 | 2.25 | **9.75** |
| Mean | | 3.10 | 1.58 | 1.13 | 2.00 | 7.80 |
| SEm ( ± ) | | 0.07 | 0.03 | 0.03 | 0.05 | 0.16 |
| CD (0.05) | | 0.19 | 0.10 | 0.08 | 0.13 | 0.45 |
| CV (%) | | 4.19 | 4.13 | 4.75 | 4.48 | 3.97 |

**Table 4: Seed mycoflora status of cowpea by blotter method.**

| Sr. No. | Place of Collection | *A. niger* | *A. flavus* | *Fus* | *Rhiz* | *Pseud* | *Xan* | Infected seed % |
|---|---|---|---|---|---|---|---|---|
| 1. | Rairakhol, Sambalpur | 4.50 | 6.75 | 3.50 | 3.25 | 5.50 | 4.25 | **27.75** |
| 2. | Kantamal, Boudh | 7.50 | 5.75 | 3.75 | 4.25 | 5.50 | 3.50 | **30.25** |
| 3. | Tarang, Deogarh | 0 | 3.50 | 2.75 | 2.25 | 5.50 | 3.25 | **17.25** |
| 4. | Jujomura, Sambalpur | 6.50 | 6.25 | 4.75 | 2.25 | 2.50 | 0 | **22.25** |
| 5. | Dunguripali, Sonepur | 6.75 | 3.50 | 3.25 | 0 | 4.25 | 1.50 | **19.25** |
| 6. | Salebhata, Balangir | 0 | 3.50 | 2.75 | 0 | 5.25 | 3.25 | **14.75** |
| 7. | Attabira, Bargarh | 5.25 | 3.50 | 3.75 | 3.25 | 4.50 | 1.75 | **22** |
| 8. | Rupra road, Kalahandi | 6.50 | 5.25 | 4.25 | 2.50 | 5.25 | 0 | **23.75** |
| 9. | Bhawanipatna, Kalahandi | 5.25 | 2.25 | 2.50 | 2.75 | 0 | 0 | **12.75** |
| 10. | Khariar, Nuapada | 6.25 | 4.50 | 4.25 | 4.75 | 5.50 | 3.25 | **28.5** |
| Mean | | 4.85 | 4.475 | 3.55 | 2.525 | 4.375 | 2.075 | 21.85 |
| SEm ( ± ) | | 0.10 | 0.10 | 0.07 | 0.06 | 0.09 | 0.06 | 0.44 |
| CD (0.05) | | 0.30 | 0.28 | 0.21 | 0.17 | 0.27 | 0.17 | 1.28 |
| CV (%) | | 4.23 | 4.28 | 4.04 | 4.56 | 4.22 | 5.59 | 4.03 |

N.B. : *Fus* means *Fusarium equiseti, A- Aspergillus, Rhizo- Rhizopus* sps., *Pseud- Pseuomonas* sps., *Xan.- Xanthomonas* sps.

**Table 5: Detection of seed mycoflora by standard blotter and PDA method.**

| Sr. No. | Place of Collection | Blotter method (%) | PDA method (%) Treated with $HgCl_2$ 0.1% | Mean Mycoflora (%) |
|---|---|---|---|---|
| 1. | Rairakhol, Sambalpur | 27.75 | 9.25 | **18.50** |
| 2. | Kantamal, Boudh | 30.25 | 11.25 | **20.75** |
| 3. | Tarang, Deogarh | 17.25 | 6.00 | **11.63** |
| 4. | Jujomura, Sambalpur | 22.25 | 8.00 | **15.13** |
| 5. | Dunguripali, Sonepur | 19.25 | 6.50 | **12.88** |
| 6. | Salebhata, Balangir | 14.75 | 6.25 | **10.50** |
| 7. | Attabira, Bargarh | 22.00 | 7.00 | **14.50** |
| 8. | Rupra road, Kalahandi | 23.75 | 8.25 | **16.00** |
| 9. | Bhawanipatna, Kalahandi | 12.75 | 5.75 | **9.25** |
| 10. | Khariar, Nuapada | 28.5 | 9.75 | **19.13** |
| **Mean fungal colonies (%)** | | **21.85** | **7.80** | |

**Table 6: Identification of seed mycoflora of cowpea.**

| Sr. No. | Place of collection | Fungi isolated |
|---|---|---|
| 1. | Rairakhol, Sambalpur | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Alternaria* sp.**,** *Rhizopus* sp., *Pseudomonas* sp. *and Xanthomonas* sp. |
| 2. | Kantamal, Boudh | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Alternaria* sp., *Rhizopus* sp., *Pseudomonas* sp. and *Xanthomonas* sp. |
| 3. | Tarang, Deogarh | *A. flavus*, *Fusarium equiseti, Pseudomonas sp., Alternaria* sp., *Macrophomina phaseolina, Rhizopus* sp., *Pseudomonas* sp. *and Xanthomonas* sp. |
| 4. | Jujomura, Sambalpur | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Alternaria* sp., *Pseudomonas* sp., and *Xanthomonas* sp. |
| 5. | Dunguripali, Sonepur | *A. flavus, A. niger, Fusarium equisetis, Macrophomina phaseolina, Pseudomonas* sp. and *Xanthomonas* sp. |
| 6. | Salebhata, Bolangir | *Fusarium equiseti, Macrophomina phaseolina, Alternaria* sp., *Pseudomonas* sp. and *Xanthomonas* sp. |
| 7. | Attabira, Bargarh | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Alternaria* sp., *Rhizopus* sp., *Pseudomonas* sp. and *Xanthomonas* sp. |
| 8. | Rupra road, Kalahandi | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Alternariasp., Rhizopus* sp., *Pseudomonas* sp. |
| 9. | Bhawanipatna, Kalahandi | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Rhizopus* sp. |
| 10. | Khariar, Nuapada | *A. flavus, A. niger, Fusarium equiseti, Macrophomina phaseolina, Alternaria* sp., *Pseudomonas* sp. and *Xanthomonas* sp. |

**Table 7: Important identifying seed mycoflora of cowpea.**

| Sr. No. | Name of fungi/bacteria | Important identifying characters |
|---|---|---|
| 1. | *Fusarium equiseti* | The fungus's mycelium is white. The hyphe is septate and branching. Both macro and micro conidia are produced by the fungus. The macro conidia have 3-7 septate cells, are somewhat curved, and have pointy ends. |
| 2. | *Macrophomina phaseolina* | It results in a blackish colony. It can produce black to brown mycelial sclerotia, as well as round to oval or round to curve shaped mycelial sclerotia. |
| 3. | *Aspergillus niger* | The mycelium is dark, with septate hyphae and niger multinucleate hyphae. Conidiophores are produced by the mycelium. The bulbuls (vecsicle) at the end of the conidiophores have 1-2 layers of sterigmata. On secondary sterigmata, conidia grow in chains. |
| 4. | *Aspergillus flavus* | The hyphe are septate and multinucleate, and the mycelium is bluish green. Conidiophores are produced by the mycelium. The bulbuls plate (vecsicle) at the end of the conidiophores has 1-2 layers of sterigmata. On secondary sterigmata, conidia develop in chains. |
| 5. | *Rhizopus* sp. | The mycelium is non-sepatate, and at some sites, it forms rhizoids (root hairs). Sporangiophores are formed directly above the rhizoids. The sporangiophores swell at the top, and sporangium forms. |
| 6. | *Alternaria* sp. | Mycelium was branched and sepated. Conodia are bottle-shaped and generally solitary, with 3-8 transverse septa and a long beak on each conidium. |
| 7. | *Pseodomonas* sp. | When grown on Kings B media, large, opaque, flat colonies with irregular edges and a characteristic fruity odour are produced by *Pseudomonas* sp. |
| 8. | *Xanthomonas* sp. | When grown on nutrient agar media, *Xanthomonas* species colonies are often mucoid, convex, and yellow. |

## CONCLUSIONS

After a thorough and critical examination of the findings of this research, the following major conclusions can be drawn: Cowpea variety Swarna of Bhawanipatna, Kalahandi district, western Odisha was found to have the highest percentage of physically pure seed (%), germination (%), viability (%), seedling vigour index-I, seedling vigour index-II, and speed of germination in farmers saved seed. Bhawanipatna collection had the lowest seed infection, but Kantamal, Boudh district had the greatest seed infection, 30.25 % in the Blotter paper methods, however it varied from 9.25 to 20.75 % in both the varieties and genotypes in PDA methods. *Macrophomina phaseolina and Alternaria* sp. both are internal seed mycoflora. In the PDA and Blotter paper methods, identification of seed mycoflora in various farmers saved seed varied from 5.75–11.25 % and 12.75–30.25 %, respectively. In the PDA method, *Aspergillus niger, Aspergillus flavus, Fusarium equiseti, and Rhizopus* sp. were identified, whereas in the blotter method, *Macrophomina phaseolina, Aspergillus flavus, Fusarium equiseti*, and *Alteranaria* sp. were detected, as well as two bacteria, *Xanthomonas* sp. and *Pseudomonas* sp. were detected in blotter method. Hence, blotter paper methods yielded a higher number of seed mycoflora than PDA methods.

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