DISEASE FREE PLANTING MATERIAL - THE KEYSTONE FOR SUCCESSFUL CULTIVATION OF BLACK PEPPER

R. Suseela Bhai* and Santhosh J Eapen *

Black pepper ostensibly called as "black gold" is the dried mature fruits of *Piper nigrum* L. coming under the family, Piperaceae. The vine is native to South India but is grown in nearly all tropical regions and currently Vietnam is leading in the export of black pepper, totalling around 35% of the world's supply. This is followed by India, Brazil, China and Sri Lanka. In India, black pepper is cultivated largely in Kerala, Karnataka and Tamil Nadu and to a limited extent in Maharashtra, North Eastern states and also in Andaman & Nicobar Islands. In India the crop is grown in about 1,31,230 hectares with a production of 55,500 tonnes annually (2015-16) and Kerala and Karnataka account for its major portion. During 2015-16, India realised an export of 28,100 tonnes worth Rs. 1,73,041.50 lakhs. Since the developmental agencies are promoting black pepper cultivation, the area under cultivation is increased to a large extent and so there is a huge demand for planting material of high yielding varieties. However, production of quality planting material is a major limiting factor in black pepper production.

Developmental agencies such as Directorate of Arecanut and Spices Development, Spices Board, State Agriculture Departments etc. are extending strong support for establishing black pepper nurseries. Healthy disease free planting material production can be ensured in a nursery by strictly following certain good agricultural practices. First and foremost is the nursery hygeine followed by adoption of timely plant protection measures for control of pests and diseases.

Plant propagation in black pepper

At present several methods are being adopted for the production of rooted planting materials in black pepper nurseries (IISR 2015).

1. **Traditional method:** In this method runner shoots are collected from high yielding and disease free healthy vines and multiplied in a nursery as two or three node cuttings. For this, initially runners from healthy mother vines are selected, then kept coiled on wooden pegs fixed at the base of the vine to prevent the shoots from coming in contact with soil which may be contaminated with residual pathogens like *Phytophthora* spp. During February-March, the runner shoots are separated from the mother vine, cut into 2-3 node pieces, leaves trimmed and planted either in nursery beds or in polythene bags filled with potting mixture (soil, sand and farm yard manure 2:1:1) and irrigated frequently. This should be done under a thatched roof in order to protect the cuttings from rain and scorching sun. The cuttings become ready

* Indian Institute of Spices Research, Marikkunnu P.O., Kozhikode, Kerala.

for planting during May-June. The major drawback of this method is the difficulty to produce large number of improved varieties released by research institutions in higher numbers since enough runner shoots of these varieties are not available (Fig 1) .

Fig. 1

2. In **rapid multiplication method** (modified from Sri Lankan method), a trench of 45 cm depth, 30 cm width of convenient length is made and filled with rooting medium containing forest soil, sand and farm yard manure in 1:1:1 ratio. Bamboo split halves or split halves of PVC pipes are fixed at 45° angle by keeping split portion facing upward on a strong support on one side of the trench. Rooted cuttings are planted in the trench at the rate of one cutting for each bamboo split. The bamboo splits are filled with rooting medium containing decomposed coir dust-farm yard manure mixture in 1:1 ratio. As the vines grows, they are tied to the bamboo split so as to keep the nodes touching the rooting medium. After reaching sufficient length, each single nodded cutting with the bunch of roots is cut and planted in polythene bags filled with fumigated or solarized potting mixture. *Trichoderma* @ 1 g and VAM @ 100 cc/kg of soil can be added to the potting mixture. The buds start developing in about three weeks and the poly bags can then be removed and kept in shade till field planting. The advantages of this method of propagation are rapid multiplication rate, well developed root system, higher field establishment and vigorous growth as a result of better root system. However, the disadvantage of the method is that if the soil mixture is get contaminated with any pathogen, the whole system will be infected and destroyed due to the spread of pathogen inoculum (Fig.2).

Fig. 2

3. **Trench method** is developed at ICAR-IISR. In this method a pit of 2.0 × 1.0 × 0.5 meter size is dug in a cool and shaded area. Single nodes of 8-10 cm length with leaf intact are taken from runner shoots from mother vines and planted in polythene bags (25×15 m of 200 gauge) filled with

nursery mixture containing sand, soil, coir dust and cow dung (1:1:1:1). The planting is done in such a way that the leaf axil exposed above the mixture. After keeping the bags in the pit, the pit should be covered with a polythene sheet. The cuttings should be irrigated at least five times a day with a rose can. Cuttings in poly bag are drenched 2-3 times with copper oxychloride (2 g/ litre). After about one month, new shoots start emerging from the leaf axil. Before two months of planting, the cuttings can be taken out of the pit and kept in a shaded place and watered twice a day. However, as in the first method, the disadvantage of this method is also the shortage of runner shoots. Also frequent watering will lead to soil-borne and air-borne infections which would be unknowingly carried to the field.

4. **Serpentine method** is an efficient system of multiplication which can be done in a nursery shed. Here rooted black pepper cuttings are planted in polythene bags containing solarized potting mixture fortified with biocontrol agent. As the plant grows and produces few nodes, small polythene bags (20 ×10 cm) filled with potting mixture may be kept under each node and the nodes may be gently pressed to the mixture using a flexible splinter to make sure their contact with the potting mixture. This is to be repeated at every node junction to induce rooting at each node. In three months the first 10 to 12 nodes would have rooted profusely and will be ready for harvest. Each node with the polythene bag is cut just below the rooted node. The rooted nodes will produce new sprouts in a week time and will be ready for field planting in 2-3 months. By this method, on an average, 60 cuttings can be harvested per mother plant in a year. Even if one plant is infected by any disease causing organism, it can be cut and removed from the lot. This is comparatively a better method for production of healthy, disease free planting materials (Fig.3).

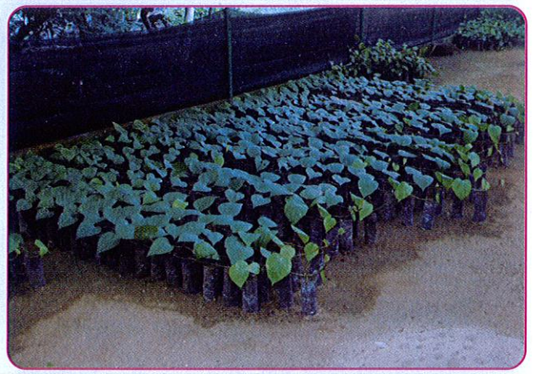

Fig. 3

5. In the **plug-tray method**, soil less nursery mixture is used for initial multiplication of black pepper runners in a modified serpentine method. First the runners are allowed to strike roots in a bed (1.5 m width 10 cm height with convenient length) filled with partially decomposed coir pith and vermicompost (75:25). The vines trail on rooting medium and strike roots at every node. After 45-60 days, about 15-20 node rooted runner is cut into single node rooted cuttings leaving the terminal five nodes and transferred to plug-trays of cell size 7.5 × 7.5 × 10.0 cm, filled with soil - less nursery mixture enriched with *Trichoderma*. Better rooting will be there if plants are

maintained under a humidity controlled greenhouse (27±2°C) with intermittent mist. The cuttings are retained in the trays for about 4-5 leaf stage for initial establishment. The established cuttings are then transferred to shade net/ naturally ventilated green house for hardening for 45-60 days and which are ready for field planting after 120-150 days (Fig.4).

Fig. 4

Fig. 5

6. **Vertical column method** is yet another novel method of enhancing quality planting material production using vertical columns with soil-less media. In this technique, orthotrops of black pepper are grown on vertical columns filled with decomposed coir pith and vermicompost @ 3:1 ratio fortified with bioagent *Trichoderma harzianum*. Single node cuttings, top shoots as well laterals or plagiotropes, which can be used for production of bush pepper, can be harvested at a time from each column. Eight to ten cuttings can be planted around each column. A hi-tech poly house is advisable for this system. The cuttings are allowed to trail on the column where each node comes in contact with the medium. It takes about 4-5 months for the cuttings to reach the top of the column. At this stage each vine will have around 20 nodes with few lateral branches. The top 5-7 nodes with lateral branches can be used as orthotropic shoots for field planting(Fig.5).

Pests and diseases in black pepper nurseries

Black pepper in nurseries is prone to infection by a number of diseases of oomycetal and fungal origin such as *Phytophthora* rot, anthracnose, basal wilt and leaf rots besides virus and nematode infections.

***Phytophthora* rot:** The disease can occur from air-borne inoculum or through soil-borne inoculum from contaminated soil. The symptoms appear on leaves as characteristic black spots with fimbriate margins which enlarge and the leaves fall off. This may serve as primary source of inoculum for root or collar infection. Infection from contaminated soil leads to collar infection as blackening which extend both upwards

and downwards. The root infection also culminates in collar infection leading to death of the plants. The disease is caused by *Phytophthora capsici*.

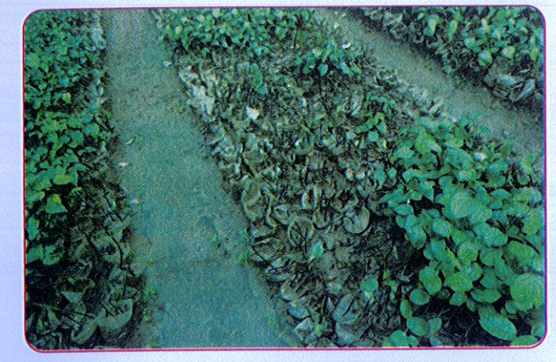

Fig. 6 (a) Symptoms of *Phytophthora* rot in the nursery

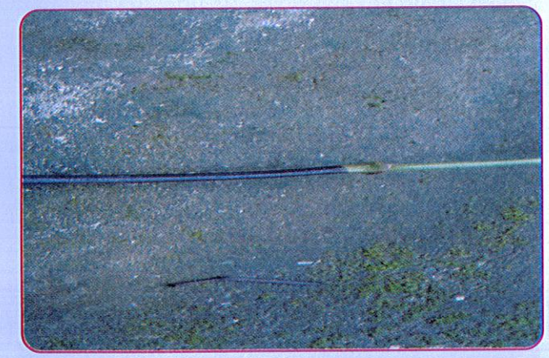

Fig. 6 (b) root infection

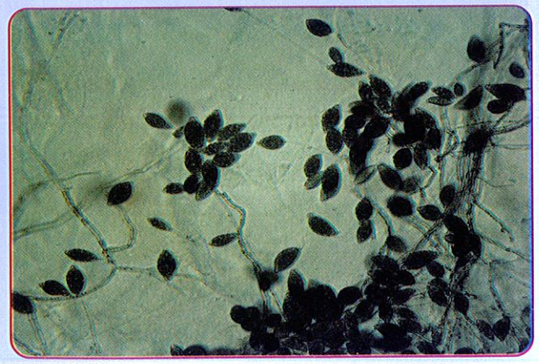

Fig.6 (c) *Phytophthora* under microscope

Anthracnose: In the nursery anthracnose disease is characterized by the appearance of yellowish brown to dark brown irregular sunken leaf spots with a chlorotic halo on the leaves. In advanced stages of infection, the lesions coalesce and leaves dry up. The disease appears during the end of monsoon. The disease is caused by *Colletotrichum gloeosporioides*. The fungus is air-borne and spreads through conidia formed in pin head like pycnidial structures formed on the infected lesions.

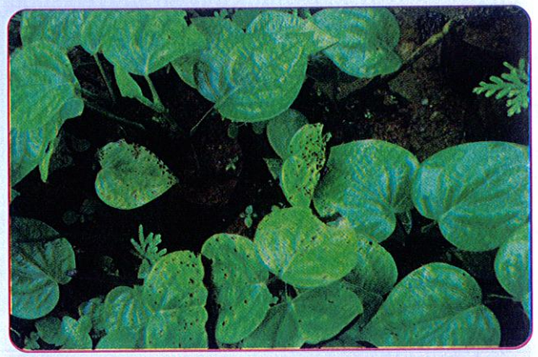

Fig. 7 Symptoms of anthracnose in a nursery

Basal wilt: Basal wilt is caused by the fungus *Sclerotium rolfsii*. The disease is mainly noticed in nurseries during June-September months. Symptoms appear as grayish lesions on stems and leaves. On leaves white mycelium are seen

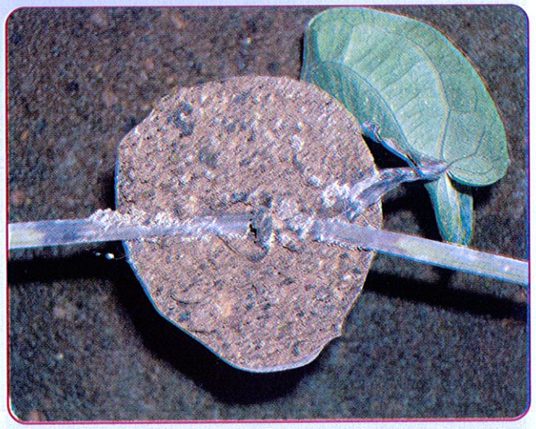

Fig. 8 Symptoms of basal wilt

at the advancing edge of the lesions. The mycelial threads later girdle the stem resulting in drooping of leaves beyond the point of infection. In advanced stages the rooted cuttings dry up. Small whitish to cream coloured grain like sclerotium beads appear on the mature lesions.

Leaf rot and leaf blight: Leaf blight is often serious in nursery during April-May when warm humid condition prevails. Infection occurs on both leaves and stem. Grayish sunken spots and mycelial threads appear on the leaves and the infected leaves are attached to one another with the mycelial threads. On stems, the infection occurs as dark brown lesions which spread both upwards and downwards. In advanced stages of infection new flushes subtending the points of infection gradually dry up and drop off. The disease is caused by *Rhizoctonia solani/ Sclerotium rolfsii*.

Fig. 9 Leaf rot symptoms (a) *Rhizoctonia solani*

Fig. 9 Leaf rot symptoms (b) *Sclerotium rolfsii*

Virus diseases: Stunted disease is one of the most important diseases affecting black pepper in the country. The disease appears as distortion, reduction in size and mottling and mosaic on the leaves along with stunting of the whole plant. In severe cases leaves become abnormally narrow and give a sickle shaped appearance. Association of two viruses namely *Cucumber mosaic virus* (CMV) and *Piper yellow mottle* (PYMV)/*Badna virus* are observed. Aphids are known to transmit CMV whereas mealy bugs like Citrus mealy bug, *Planococcus citri* (Risso), and striped mealy bug *Ferrisia virgata* are known to transmit the Badna virus associated with stunted disease. The disease is inadvertently carried from nurseries to plantation through the use of infected planting material.

Fig. 10 Virus sysmtoms in a black pepper nursery

Nematodes in the nursery: Plant parasitic nematodes like *Meloidogyne incognita* (root-knot nematode) and *Radopholus similis* (lesion nematode) are major problems in black pepper nursery which are also carried through planting material into plantations. *M. incognita* causes typical galls or knots on the roots and elongated

swelling on thick primary roots whereas *R. similis* causes necrotic lesions on white feeder roots.

Good Agricultural Practices in black pepper nurseries

The National Horticulture Mission (NHM) has specified the infrastructure required for setting up of a 'Model Horticulture Nursery' which is notified and published by ICAR in the "Handbook of Seed and Planting Material Testing Manual for Horticultural Crops". The existing rules framed under Horticulture Nursery (Regulation) Acts, provide for record keeping in such nurseries. Since the nursery activity of black pepper is for a short span of 3-4 months, it is better to follow an integrated management strategy to protect the plants from infection caused by various type of pathogens. The infection may occur mainly from contaminated soil/potting mixture besides the air borne inoculum like *Colletotrichum* sp. or other foliar pathogens like *R. solani* and *S. rolfsii*. For the integrated management of nursery and for the production of disease-free and healthy planting material the following good agriculture practices have to be followed.

1. Selection of mother vines - Only healthy vines of high yielding improved varieties or promising farmer's varieties alone should be selected as mother vines. If needed, they should be indexed for the presence of any viruses.

2. Disinfestation of potting mixture - can be achieved either by fumigation, solarization or by steam sterilization. Among these, soil solarization is the most economical and efficient method.

3. Fortification of potting mixture - The disinfested potting mixture should be fortified with suitable biocontrol agents like *Trichoderma, Pseudomonas, Pochonia* etc.

4. Nursery manangement - recommended dosages of fertilizers, pesticides or biocontrol agents should be applied at regular intervals. The plants should be watered every day and never allow excess moisture in the nursery.

5. Nursery hygeine - The plants in the nursery should be closely monitored for incidence of pests, diseases etc. The infected plants should be removed immediately from the nursery and destroyed.

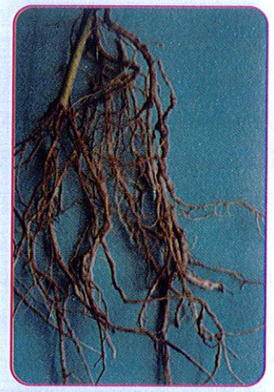

Fig. 11 Nematode symptoms in black pepper cuttings (a) Root lesion due to *R.similis*

Fig. 11 Nematode symptoms in black pepper cuttings (b) Root galls due to *M.incognita*

Establishment of model nurseries

In order to demonstrate the good agriculture practices and for creating awareness and confidence among farmers, ICAR-IISR has taken an initiative to establish a few model nurseries under the Area Wide Integrated Pest Management (AWIPM) project supported by Kerala State Agriculture Department. A non-chemical, bio-intensive management strategy was adopted in these nurseries. A shed of size 24 m x 20 m with white polythene sheet (100 microns) roofing was erected. Nursery mixture was prepared by mixing soil, sand and FYM in 1:2:1 ratio and sterilized by solarization. For this, the nursery mixture prepared was made into beds of size 3 m x 1 m in a place where there is direct exposure to sunlight. The bed was watered thoroughly and covered with white polythene sheet of 100 microns and sealed air tight and kept for 40 -50 days. After solarization is over, single node virus free rooted cuttings (virus indexed plants) of different released varieties of black pepper, raised under insect proof conditions at ICAR-IISR, were used as the nucleus planting material.

Biocontrol agents such as *Trichoderma harzianum*, *Pochonia chlamydosporia* and actinomycetes strains viz. *Streptomyces tauricus* (strain Act 9), *Streptomyces* sp. (strain Act 5) and *Ketosatospora setae* (strain Act 2) (Bhai *et al.*, 2016) were incorporated into solarized nursery mixture in different combinations and filled in polythene bags of size 15 cm x 10 cm. Metalaxyl mancozeb (0.125%) and carbosulphan 0.1% were incorporated @1 L in the potting mixture as done for biogents. Single node virus free (indexed) rooted cuttings of varieties were planted in these polybags and were allowed to grow by serpentine method (Thankamani *et al.*, 2005). Irrigation was provided only on alternate days and nursery was maintained hygienically with regular monitoring. When the number of rooted plants in the serpentine reached around 10-15, the rooted middle cuttings were cut and separated leaving three plants at the tip and nucleus material at the end (which were allowed to grow further). The plants were kept for establishment till they reach a 3-4 leaf stage.

Nine months after starting the serpentine method of propagation, the variety IISR Malabar Excel and IISR Thevam produced the maximum number of plants from a single node cutting treated with *T. harzianum* + *P. chlamydosporia* (T1) followed by Act 2+9 and Act 5+9. On an average 6-7 plants / month / cutting were obtained on

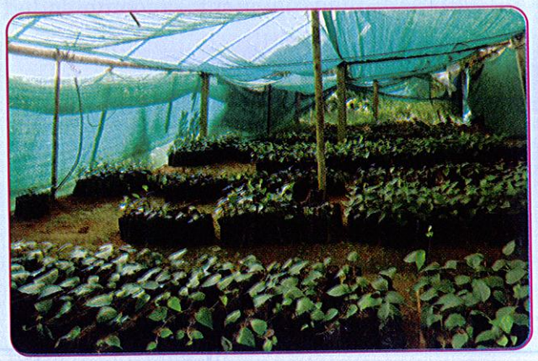

Fig. 12. a & b : A view of the Nursery at Venappara in Omasserry under Thamarassery Panchayat of Kozhikode District

treating with these bioagents while it was only 4 plants/month/ cutting in control. In case of IISR Girimunda also, the performance was almost same in all the three bioagent combination when compared to control. Only IISR Shakthi showed comparatively lesser performance with bioagent combination. The rate of multiplication in chemical treatment was comparatively lesser when compared to control and bioagent treatment.

The biomass of rooted cuttings was significantly superior with actinomycetes combination (Act 2+9) and was on par with that of *T.harzianum* and *P.chlamydosporia* treatment. The height of the plants, number of roots and root biomass were significantly higher in bioagent treated compared to chemical treatment and absolute control. There was no incidence of soil borne infections caused by *P. capsici*, *S. rolfsii* , *C. gloeosporioides* or nematodes or even virus (the common diseases otherwise observed in nurseries) in these nurseries probably because of GAP like solarization of potting mixture, restricted irrigation and improved nursery hygiene. In general nutrient status of the soil considerably increased in biocontrol combination due to their growth promoting traits like production of IAA or through siderophore production etc. (Bhai *et al.*, 2016). The nutrients available in the fortified mixture was found sufficient for the growth of rooted cuttings till their planting.

Amending soil with *Trichoderma harzianum* and *Pseudomonas fluorescens* has already been reported (Thankamani *et al.*, 2005). But use of *Trichoderma harzianum* together with *Pochonia chlamydosporia* or with antibiotic producing actinomycetes for production of healthy, rooted cuttings of black pepper is a first time report. So, solarisation of nursery mixture followed by fortification with either *Trichoderma* + *Pochonia* or actinomycetes combinations (2+9 or 5+9) is strongly recommended for the production of healthy planting material in black pepper. Besides, by following this GAP in black pepper nursery, the farmers will be able to earn a sizable income too by sale of rooted cuttings. Mr. George Nedumkallel at Venappara produced more than 15,000 plants from 100 nucleus planting material of four varieties within a short span of ten months and earned an income of Rs.3 lakhs.

Conclusion

Production of disease free quality planting material is a major limitation in establishing black pepper plantations in the country and there is a huge demand for good quality planting material. Establishment of such nurseries to ensure the availability of good quality, disease free, planting material of desired high yielding variety will have a tremendous impact on production, productivity and quality of black pepper. This successful intervention is strongly recommended to all agencies involved in black pepper nurseries in the country. The procedure is simple and highly suitable for the production of disease free planting material of high yielding varieties and also farmers varieties to meet the increasing demand for planting material.

Acknowledgement:

The authors are thankful to Department of Agriculture for funding and Mr. George, Nedumkallel, farmer at Omasserry, for active cooperation and involvement.

References

Devasahayam, S., John Zacharaiah ,T., Jayashree, E., Kandiannan, K., Prasath, Santhosh J Eapen, Sasikumar, B., Srinivasan, V. and Suseela Bhai, R. 2015 *Black pepper - Extension pamphlet* ICAR Indian Institute of Spices Research, Kozhikode

Suseela Bhai, R., Lijina, A., Prameela,T.P., Krishna, P. B. and Anusree Thampi, 2016 Biocontrol and growth promotive potential of *Streptomyces* spp. in black pepper (*Piper nigrum* L.) *J. Biological Control*, 30 (3): 177-189, 2016, DOI: 10.18311/jbc/2016/15592

Thankamani, C.K, Sreekala K. and Anandaraj M. 2005. Effect of *Pseudomonas fluorescens* (IISR-6) and *Trichoderma harzianum* (P-26) on growth of black pepper (*Piper nigrum* L.) in the nursery. *J. Spices and Arom. Crops* 14 (2): 112-116.

Thankamani, C. K., Dinesh, R.., Eapen, S. J., Kumar, A., Kandiannan, K and Mathew, P.A. 2007. Effect of solarized potting mixture on growth of black pepper rooted cuttings (*Piper nigrum* L.) in the nursery. *J. spices and arom. crops*, (Supplement): 103-10

Handbook of Agriculture, New Delhi, ICAR 2015

****