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Evaluation of bioagents against *Colletotrichum capsici* caused anthracnose disease of yam (*Dioscorea alata* L.)

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Abstract
Three bioagents were evaluated for management of anthracnose disease of yam by sett treatment, soil application and spray methods with untreated control. Among the seven treatments, higher germination (86.46%) and lowest disease intensity (19.61%) was observed in Sett biopriming with *Trichoderma harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) as compare to rest of the treatments. While, lowest germination (76.04%) and highest disease intensity (45.57%) was recorded under Control. Effect of treatments on the yam tuber yield was also found significantly superior as compared to the control. The results showed that the maximum yield was obtained in Sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (7651.75 kg ha^{-1}) which was at par with Sett biopriming with *T. viride* @ 10 g/kg + 2 spray of *T. viride* (0.5%) (7343.11 kg ha^{-1}). While, lowest yam tubers yield (5594.14 kg ha^{-1}) was recorded in control.

Keywords: *In vivo*, bioagents, *Colletotrichum capsici*, anthracnose, *Dioscorea alata*

Introduction
The yam is a common name for some species in the genus *Dioscorea* (family: Dioscorcaceae). These perennial vines are cultivated for consumption of starchy tubers in Africa, Asia, Latin America and Oceania. Yam is considered to be originated from the Indo-Burmese region of South East Asia and consisted of about 600 species which are mostly tropical in distribution (Thamburaj and Singh, 2005) [9]. Yam with average world productivity of 9.2 t ha^{-1} are cultivated globally in 4.4 million hectare's, with production of 40 million tonnes, while in India, the crop covers 30,000 ha area with 80,000 MT (0.8 million) production and has an average productivity of 28 t ha^{-1} (Abraham *et al.* 2006). The major yam producing states in India includes Gujarat, Maharashtra, Orissa, Rajasthan, Kerala, West Bengal, Bihar and Assam. Two Asiatic yams, *viz. Dioscorea alata* Linn (greater yam) and *Dioscorea esculenta* (Lour.) Murkill (lesser yam) are the major food of the Indians. The yams exploited for pharmaceutical purposes are non-edible, (Thamburaj and Singh, 2005) [9]. Yams form staple diet in many parts of Western Africa. The processing and consumption are still by conventional methods. The conventional processing techniques are boiling, roasting, frying or conversion to fufu. Fufu (a cooked and mashed yam tuber) is an important product made from yam in Western and Central Africa. Wafers, crepes and biscuits made out of yam tubers were found to have good acceptability. Traditionally in many Indian families yam tubers are consumed after cooking and peeling. Yam are a valuable source of carbohydrate to the people of the tropical and subtropical Africa, Central and South America, parts of Asia, the Caribbean and Pacific Islands (Coursey, 1967; Adelusi and Lawanson, 1987) [3, 2]. *D. alata* tubers are peeled and cooked or used as vegetable. The 100 g edible portion of yams contains 8 mg calcium, 28 mg Phosphorous, 1.1 mg Iron, 5 mg Vitamin A, 0.10 mg Thiamine, 0.04 mg Riboflavin, 0.5 mg Niacin and 6 mg Ascorbic acid (Tindall,1983). Many fungal and viral diseases have been observed in edible yams at various stages of growth and production. Among the fungal diseases, anthracnose, (*Colletotriehum gloeosporioides* Penz. and Sacc.), Cercospora leaf spot, Curvularia leaf spot (*Curvularia eragrostides* (Henn.) Meyer), leaf blight (*Pestalotia* sp.) and dry rot **(***Botryodiplodia theobromae* Pat., *Penicillium oxalicum* Currie and Thom, *Penicillium italicum* Wehmer), soft rot *(Rhizopus nigricans* Ehr., *Sclerotiumum rolfsii* Sacc.) and bacterial disease *viz.*, wet rot *(Erwinia caratovora* sub. sp. *caratovora* Jones) in storage are important ones. In case of viral disease, yam mosaic disease is reported. This disease is caused by an aphid-transmitted potyvirus that infects several species of Dioscorea, particularly *D. alata* L., *D. cayenensis* Lam, *D. rotundata* Poir and *D. trifida* L. (Mantell, 1980; IITA, 1993) [5, 4].

The anthracnose of yam is caused by *Colletotrichum gloeosporioides* Penz. and Sacc. was first reported in Rajasthan in devastating form and caused 70-80 per cent loss in yield under favourable climatic conditions. This disease is now widespread in India and occurs every year on all *Dioscorea* spp. but in severe form only in *D. alata*. The initial symptoms appeared as brown pin-head like spots on the leaves and in advanced stages leaves and stems are completely blighted and dried up (Thamburaj and Singh, 2005) [9]. The anthracnose disease was observed in severe form on the horticultural farm of the Navsari Agricultural University, Navsari, in the year 2007 on the *Dioscorea alata* and *Colletotrichum capsici* (Syd.) Butler and Bisby was observed to be constantly associated with the disease (Mehetre, 2009). Since then the disease was found in m oderate form. Again this disease was found in October, 2015. So, the problem was undertaken to generate scientific information and for finding the recent management measures.

Materials and Method

The experiment was conducted at College research farm, Navsari Agricultural University, Navsari during 2016-17 and 2017-18 using Randomised Block Design (RBD) with seven treatments *viz.* T1-Sett biopriming with *Trichoderma viride* @ 10 g/kg, T2-Sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* (0.5%), T3-Sett biopriming with *T. harzianum* @ 10 g/kg, T4-Sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%), T5-Sett biopriming with *Pseudomonas fluorescence* @ 10 ml/kg, T6-Sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%), T7-Control (without treatment) and three replications on yam crop cultivar NAUDA-1. Yam setts were coated with the talc based and soaked in liquid based formulations of all bioagents and mixed thoroughly to provide uniform coating. The setts were dried in shade and stored at 25±2 °C for 8 hours and were sown in experimental plot. Two sprays of bio agents were given, first at the time of initiation of the disease and second at 15 days after first spray. The efficacy of these treatments was compared with the control plot. Five plants from each treatment were tagged for recording the observations and the disease intensity was recorded using 0-6 scale (Scale 0: Nil, scale 1: 0.1-10% infection, scale 2: 10.1-20% infection, scale 3: 20.1-30% infection, scale 4: 30.1-40% infection, scale 5: 40.1-50% infection and scale 6: >50% infection) as described by Palarpawar and Ghurde (1989) [7] and per cent disease intensity (PDI) was calculated by following formula:

$$\text{PDI} = \frac{\sum \text{of ratings of infected leaves observed}}{\text{No. of leaves observed X Maximum disease score}} \text{ X } 100$$

The observations on germination per cent were recorded as per the formula mentioned below, tuber yield per plot was also recorded and was converted on hectare. The generated data were statistically analyzed and presented.

$$\text{Germination (\%)} = \frac{\text{Total no.of setts germinated}}{\text{Total no.of setts sown}} \text{X} 100$$

Results and Discussion

A field experiment with seven treatments was conducted during the year 2016-17 and 2017-18 to study the efficacy of different bioagents that would minimize the anthracnose disease intensity thereby maximizing the yield of yam. The results obtained on per cent disease intensity and yields were presented (pooled data of both year 2016-17 and 2017-18) in Table-1. There was significant difference among the different treatments with respect to PDI and yield.

The data presented in Table-1 revealed that all the treatments were found non significant to average per cent germination of yam sett. Among seven treatments, higher germination was observed in sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (86.46 %). The next best treatments in order to average per cent germination were sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* (0.5%) (83.34%), sett biopriming with *T. harzianum* @ 10 g/kg (81.25%), sett biopriming with *P. fluorescence* @ 10 ml/kg (80.21), sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%) (80.21%) and sett biopriming with *T. viride* @ 10 g/kg (79.17 %) were found increased over lowest germination in control (76.04%).

Before sprays, all the treatments were found non significant and also superior in their efficacy as compared to control against the anthracnose disease of yam. Among seven treatments, the lower disease intensity was observed in sett biopriming with *T. harzianum* @ 10 g/kg (4.75%) and it was found superior then rest of the treatments. The next best treatments in terms of less disease intensity were sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (4.93%), sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* (0.5%) (5.18%), sett biopriming with *T. viride* @ 10 g/kg (5.53%), sett biopriming with *P. fluorescence* @ 10 ml/kg (5.53%) and sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%) (5.56%) which were found superior then control (7.40%).

After 1st sprays, there was overall negligible increase in disease intensity. All treatments were found significantly superior in their efficacy as compared to control. Among the seven treatments, sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (10.12%) which was at par with sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* (0.5%) (10.13%). Next best treatment was sett biopriming with *T. viride* @ 10 g/kg (13.30%) which was followed by and sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%) (13.19%). Sett biopriming with *T. harzianum* @ 10 g/kg (13.41%) and sett biopriming with *P. fluorescence* @ 10 ml/kg (15.36%) were also found good. While, highest disease intensity was observed in control (17.28%).

After 2nd sprays, there is slight increase in disease intensity. All the treatments were found significantly superior in their efficacy as compared to control. Significantly lower disease intensity was observed in Sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (13.25%) which was followed by sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* (0.5%) (15.81%). Next best treatments were sett biopriming with *T. harzianum* @ 10 g/kg (21.25%), sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%) (23.15%) and sett biopriming with *T. viride* @ 10 g/kg (23.45%). Sett biopriming with *P. fluorescence* @ 10 ml/kg (25.77%) was found comparatively less effective. While, highest disease intensity was observed in control (28.20%).

Before harvesting, disease intensity increased more. All the treatments were found significantly superior in their efficacy as compared to control. Significantly less disease intensity was observed in sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (19.61%) which was at par with sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* (0.5%) (21.01%). Next best treatments in order were

sett biopriming with *T. harzianum* @ 10 g/kg (26.22%) and sett biopriming with *T. viride* @ 10 g/kg (27.03%). Sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%) (32.65%) and sett biopriming with *P. fluorescence* @ 10 ml/kg (35.03%) were found comparatively less effective against anthracnose of yam. While, highest disease intensity was observed in control (45.57%).

Effect of treatments on tuber yield of yam was also found significantly superior as compared to control. The results showed that the maximum yield was obtained in sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%) (7651.75 kg ha^{-1}) which was at par with sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* 0.5% (7343.11 kg ha^{-1}). It was followed by sett biopriming with *T. harzianum* @ 10 g/kg (6995.88 kg ha^{-1}), sett biopriming with *T. viride* @ 10 g/kg (6597.22 kg ha^{-1}), sett biopriming with *P. fluorescence* @ 10 ml/kg (6288.58 kg ha^{-1}) and sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%) (6082.80 kg ha^{-1}). While, lowest yam tubers yield (5594.14 kg ha^{-1}) was recorded in control.

Table 1: Field evaluation of various bioagents for the management of anthracnose of yam during 2016-17 and 2017-18 (pooled)

Sr. No.	Treatment	Germination (%)	Per cent Disease Intensity				Yield (kg ha^{-1})
			1 day before spray	15 days after 1st spray	15 days after 2nd spray	Before 15 days of harvesting	
1	Sett biopriming with *Trichoderma viride* @ 10 g/kg	63.19* (79.17)**	13.57 (5.53)	21.36 (13.3)	28.93 (23.45)	31.31 (27.03)	6597.22
2	Sett biopriming with *T. viride* @ 10 g/kg + 2 sprays of *T. viride* 0.5%	66.95 (83.34)	13.11 (5.18)	18.51 (10.13)	23.41 (15.81)	27.26 (21.01)	7343.11
3	Sett biopriming with *T. harzianum* @ 10 g/kg	65.15 (81.25)	13.05 (4.75)	21.44 (13.41)	27.43 (21.25)	30.78 (26.22)	6995.88
4	Sett biopriming with *T. harzianum* @ 10 g/kg + 2 sprays of *T. harzianum* (0.5%)	68.86 (86.46)	12.76 (4.93)	18.53 (10.12)	21.31 (13.25)	26.26 (19.61)	7651.75
5	Sett biopriming with *Pseudomonas fluorescence* @ 10 ml/kg	64.11 (80.21)	13.57 (5.53)	23.06 (15.36)	30.50 (25.77)	36.27 (35.03)	6288.58
6	Sett biopriming with *P. fluorescence* @ 10 ml/kg + 2 sprays of *P. fluorescence* (0.6%)	64.11 (80.21)	13.60 (5.56)	21.25 (13.19)	28.74 (23.15)	34.83 (32.65)	6082.82
7	Control (without treatment)	60.76 (76.04)	15.43 (7.40)	24.54 (17.28)	32.06 (28.20)	41.28 (43.57)	5594.14
S.Em ±		3.61	0.61	0.53	0.41	0.44	324.32
CD at 5%		NS	NS	1.63	1.18	1.28	647.8
C.V %		9.68	7.78	4.31	3.84	2.33	8.98

**Figures in parenthesis are original values. *Figures outside parenthesis are arcsine transformed value

Several workers have reported the effectiveness of bioagents in control of diseases caused by *Colletotrichum* spp. Watve *et al*. (2009) [11] found that all the bio control agents *in vitro* were antagonist of *Colletotrichum gloeosporioides*, maximum per cent inhibition in colony diameter of the test fungus was obtained by *Trichoderma harzianum* (83.33%) followed by *Trichoderma viride* (77.78%) and *Bacillus subtilis* (77.78%) when the test fungus was at centre. In the *in vivo* assay, *Trichoderma harzianum* recorded lowest leaf spot intensity (24.74 PDI) with per cent disease reduction of 20.28, while *Trichoderma viride* recorded leaf spot intensity (15.68 PDI) with per cent disease reduction of 18.48 as compared to carbendazim (0.1 %) which recorded highest per cent reduction (76.65%) of leaf spot intensity (7.38 PDI). Srinivas *et al.* (2006) [8] reported treatment of chilli seeds with pure culture of *P. fluroscense* at the rate of $1x10^8$ cfu/g which increased germination by 18% whereas talcum powder formulation of the same increased germination by 13% and 15% at the rate of 5 g/kg and 10 g/kg seeds, respectively. *T. harzianum* pure culture increased germination by 10% and the formulation of same increased germination by 5% and 7% at the rate of 5 g/kg and 10 g/kg seeds, respectively.

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