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Combined Effect of Insecticide and Fungicides on Seed Quality of Okra

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ABSTRACT Seeds of okra variety Arka Anamika were treated with an insecticide Goucho (Imidacloprid) in combination with fungicides like Anucop-5- (Copper oxychloride), Dithane M-45 (Mancozeb 75%), Vitavax (Carboxin 75%), Captan (Kohicap 50%), Bavistin (Carbendazim 50%) and their efficacy was tested against some seed-borne fungal diseases. Among them Goucho + Bavistin, Goucho + Bavistin + Captan, Goucho + Anucop + Captan, Goucho + Vitavax + Anucop were superior in improving the crop stand both in greenhouse and field. These combinations increased the biomass of plants, total number of leaves and fruits. Apart from these, total number of seeds per fruit, 1000 seed weight and ascorbic acid content also increased. In contrast, these combinations reduced the incidence of seed mycoflora and indirectly enhanced the seed germination percentage and vigour index of the seedlings.

Key words: Insecticide, fungicide, okra, seed-borne fungal pathogen, seed yield, quality

Okra (*Abelmoschus esculentus* (L) Moench) is an important summer/rainy season vegetable crop, which is attacked by many insects and pathogens, resulting in considerable crop losses. Such crop losses may be due to various reasons among which seed-borne fungal pathogens play a major role. To overcome these losses, seed treatment with chemical fungicides was the practical solution. Okra seeds treated with insecticides improved the percentage of germination, vigour index, quality and quantity of seeds by reducing the seed mycoflora [1].

Treatment of seeds with fungicides, insecticides in combination has become a routine practice by all seed agencies involved in marketing. Seed dressing is the most practical method for controlling seed and seedling diseases which in turn improves the vegetable crop. Heavy losses caused by seedborne pathogens have been observed in various crops. Hence, in the present investigation emphasis have been given to deal with the association of mycoflora, their role in pre and post emergence losses as well as ways to control them.

MATERIALS AND METHODS

Seed samples of okra viz., Arka Anamika, Arka Abhy, Pusa Sawani and few local varieties were collected from Indian Institute of Horticulture Research and Private Seed companies at Bangalore. Following the procedures of ISTA, seed samples were screened for their associated mycoflora [2]. Five different fungicides in combination with an insecticide Goucho (Imidacloprid) were used for seed treatment. Insecticide Goucho (Imidacloprid) 7g/kg and fungicides like Anucop-50 (Copper oxychloride) 3 g/kg, Dithane M-45 (Mancozeb 75%) 3 g/kg, Vitavax (Carboxin 75%) 3 g/kg, Captan (Kohicap 50%) 3 g/kg, Bavistin (Carbendazim 50%) 2 g/kg were used for seed treatment in combinations. The combinations of insecticide with fungicides were Goucho + Vitavax (0.2+0.06%), Goucho + Bavistin (0.2+0.06%), Goucho + Captan (0.2+0.10%), Goucho + Dithane M-45 (0.2+0.06%), Goucho + Anucop (0.2+0.10%), Goucho + Bavistin + Captan (0.2+0.06+0.10%) Goucho + Captan + Dithane M-45 (0.2+0.06+0.10%), Goucho + Anucop + Captan (0.2 + 0.10 + 0.10%), Goucho + Vitavax

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+ Anucop (0.2 + 0.06 + 0.10%). In seed treatment, slurry of these combinations were employed. Treated seeds were plated on moistened blotter sheets, then the paper towels were rolled, incubated for 10 days under standard conditions. On 10^{th} day of incubation, percentage of seed germination and root, shoot length of the seedlings were measured and the vigour index was calculated [3].

Simultaneously, treated seeds were sown in the greenhouse and in field for the assessment of percentage seedling emergence and pre-emergence mortality. Both in greenhouse and field conditions untreated seeds sown under similar conditions were served as corresponding controls. In all the cases, parameters like plant height, number of leaves/plant, number of fruits/plant, number of seeds/ fruit, mean fruit weight, girth and seed density were recorded.

For the purpose of ascorbic acid estimation fresh fruits were ground into a paste using 2 ml of distilled water in a pestle and mortar. 100 mg of paste was used for extraction and estimation of ascorbic acid based on dinitrophenyl hydrazine (DNPH) method [4]. For this purpose, standard ascorbic acid solution was prepared using 5 per cent trichloro acetic acid (TCA) and this was taken in 6 clean test tubes ranging from 0-1 ml, solution of unknown concentration was taken in another test tube. The volume of standard solution was made up to 1 ml by adding 5 per cent TCA, 1ml of DNPH was added to each tube. The solutions in tubes were boiled in water bath for 15 minutes and then cooled to room temperature. 8 ml of 65 per cent cold H_2SO_4 was added carefully to make up the volume to 10ml. The test tubes were kept at room temperature for about half an hour. The optical density was read at 540nm, using the data, standard curve was prepared and the total amount of ascorbic acid was determined.

RESULTS AND DISCUSSION

Data provided in Table 1 revealed varied occurrence of fungi in okra seeds obtained from treated plants. Fungi like *Macrophomina phaseolina, Fusarium solani, Fusarium moniliforme,* Actinomycete species, *Botryodiplodia theobromae, Colletotrichum dematium, Trichothecium roseum* and *Rhizoctonia solani* were drastically reduced under the treatment of imidacloprid + Kohicap, Imidacloprid + Carbendazim and Imidacloprid + Copper oxychloride, Imidacloprid + Carbendazim + Kohicap, Imidacloprid + Copper oxychloride, Imidacloprid + Kohicap + Mancozeb. These combinations improved seed germination, seedling vigour and thus stood superior over other treatments including control. Both in greenhouse and field conditions, these chemical combinations reduced the pre-emergence mortality to a greater extent. Other combinations like imidacloprid + Carboxin, Imidacloprid + Mancozeb, Imidacloprid + Kohicap + Mancozeb were least effective with respect to the incidence of seed mycoflora, seed germination, vigour index, emergence rate and pre-emergence mortality (Table 1 and 2).

Qualitative and quantitative assessment of okra in greenhouse and field conditions remained parallel to each other with respect to biomass of root, shoot and number of leaves. Further observations included the number of fruits per plant, average length of fruits, number of seeds per fruit, seed density and ascorbic acid content indicated the favourable effects due to combinations of Imidacloprid + Kohicap, Imidacloprid + Copper oxychloride and Imidacloprid + Carbendazim (Table 3).

Further, data on qualitative and quantitative analysis of seeds obtained from the treated plants, maintained under similar treatments both in greenhouse and field conditions were recorded and tabulated in Table 4. As per the data, Imidacloprid + Carbendazim, Imidacloprid + Copper oxychloride effectively controlled the mycoflora compared to their respective control and other combinations (Table 4).

Efficacy of the fungicides varies depending upon the type of the host and ecological conditions that prevailed during the plant growth. Varied effect of the fungicides probably depends upon the nature and type of the chemicals [5, 6].

Differential behaviour of fungicides in controlling fungi also depends upon the rate of penetration into the tissues and the site of infection. Effective fungicides might have translocated into the deeper tissues to reach the

Table 1. Synergestic effect of insecticide and fungicides on seed mycoflora of okra

Fungi	Control	% incidence of seed mycoflora under different combinations of insecticide and fungicides								
		G+V	G+B	G+C	G+D	G+A	G+B+C	G+C+D	G+A+C	G+V+A
Actinomycete species	30	2	0	0	1	0	0	0	0	0
Alternaria alternata	32	4	0	0	1	0	0	0	0	0
Aspergillus flavus	5	4	2	0	2	0	0	0	0	0
Aspergillus fumigatus	4	2	0	0	0	0	0	0	0	0
Aspergillus columnaris	5	2	1	0	4	0	0	2	0	0
Aspergillus niger	5	0	0	0	2	0	0	2	0	0
Botryodiplodia theobromae	5	0	0	0	0	0	0	0	0	0
Chaetomium globosum	2	0	0	0	0	0	0	1	0	0
Colletotrichum dematium	2	0	0	0	0	0	0	0	0	0
Fusarium moniliforme	75	4	1	0	1	0	0	2	0	0
Fusarium solani	20	0	0	0	0	0	0	1	0	0
Macrophomina phaseolina	72	2	1	0	1	0	0	1	0	0
Rhizoctonia solani	2	0	0	0	0	0	0	0	0	0
Rhizopus stolonifer	10	0	0	0	1	0	0	0	0	0
Trichothecium roseum	1	0	0	0	0	0	0	0	0	0

Data based on 400 seeds; G = Goucho; B = Bavistin; D = Dithane M-45; V = Vitavax; C = Captan; A = Anucop.

site of infection. The fungicides on interaction with the metabolites produced with in the tissues may form some complex compounds which may become toxic to fungi or other wise it may also interfere with the uptake of nutrients by fungi from the host tissues. The increase in germination and vigour of seedlings under certain fungicidal treatment is probably due to increase in the production of phenols, reducing sugars or total sugars. This is in conformity with the findings in which similar effects were described in wheat plants [7]. It is also known that some of the fungicides induce resistance in plants by enhancing the concentration of phenolics and carbohydrates. In support of this, higher concentration of total phenols and carbohydrates in resistant varities than in susceptible varities of groundnut plants having tikka diseases have already been reported [7]. The antifungal property of systemic fungicides were reported to be due to interference of sterols with fungi [6, 8]. Many workers have reported the combined effects of Bavistin, Benomyl, Vitavax, Thiram and Dithane M-45 against plant pathogenic fungi [9-11]. Fungicides in combination may serve both as contact and systemic ones. Hence, they might have resulted in the reduction of fungal flora to a greater extent. So, the present study suggested the usage of fungicides in combination than individual chemicals. Gailewad *et al.* [13] reported that carbofuron applied for seed treatment did not affect the germination of okra seeds. It was also found that higher concentration of carbofuron (7%) increased the vigour of seedlings in cotton [14].

Table 2. Response of okra due to combined effect of insecticide and fungicides

Treatment	Concentration of chemicals(%)	Seed germination (%)	MRL±SE (cm)	MSL±SE (cm)	Vigour index	Greenhouse condition		Field condition	
						Seedling emergence (%)	Pre-emergence mortality (%)	Seedling emergence (%)	Pre-emergence mortality (%)
Goucho+Vitavax	0.2+0.06	60.5±0.1^{e}	3.5±0.1	8.1±0.4^{d}	695.5±17.5^{d}	58.5±0.5^{g}	41.5±0.5^{d}	59.0±0.0^{f}	41.0±0.0^{c}
Goucho+Bavistin	0.2+0.06	70.5±0.5^{b}	4.8±0.1	9.4±0.4^{b}	994.0±21.0^{b}	80.5±0.5^{b}	19.5±0.5^{i}	80.5±0.5^{b}	19.5±0.5^{g}
Goucho+Captan	0.2+0.10	59.0±0.1^{f}	4.4±0.1	8.2±0.3^{d}	737.5±17.5^{c}	60.5±0.5f	39.5±0.5^{e}	59.5±0.5^{f}	40.5±0.5^{c}
Goucho+Dithane M-45	0.2+0.06	61.5±0.5^{d}	3.8±0.0	6.8±0.2^{g}	648.5±20.5^{e}	55.5±0.5^{f}	44.5±0.5^{c}	58.5±0.5^{f}	41.5±0.5^{c}
Goucho+Anucop	0.2+0.10	60.5±0.5^{e}	4.0±0.0	6.5±0.2^{h}	632.0±14.0^{e}	52.5±0.5^{i}	47.5±0.5^{b}	55.0±0.0^{g}	45.0±0.0^{b}
Goucho+Bavistin+Captan	0.2+0.06+0.10	75.5±0.5^{a}	5.5±0.5	10.2±0.4^{a}	1163.0±0.0^{a}	82.5±0.5^{a}	17.5±0.5^{j}	85.5±0.5^{a}	14.5±0.5^{h}
Goucho+Captan+ Dithane M-45	0.2+0.10+0.06	65.5±0.5^{c}	4.6±0.5	7.2±0.2^{e}	770.0±22.0^{c}	64.5±0.5^{d}	35.5±0.5^{g}	67.5±0.5^{d}	32.5±0.5^{e}
Goucho+Anucop+Captan	0.2+0.10+0.10	62.5±0.5^{d}	5.0±0.1	7.0±0.2^{f}	743.5±24.5^{c}	62.5±05^{e}	37.5±0.5^{f}	64.5±0.5^{e}	35.5±0.5^{d}
Goucho+Vitavax+Anucop	0.2+0.06+0.10	64.5±0.5^{c}	3.2±0.2	9.1±0.4^{c}	783.5±28.5^{c}	70.5±0.5°	29.5±0.5^{h}	70.5±0.5^{c}	29.5±0.5^{f}
Control		50.5±0.5^{g}	2.6±0.1	5.2±0.2^{i}	391.5±16.5^{f}	50.0±0.0	50.0±0.0^{a}	50.0±0.0^{h}	50.0±0.0^{a}

Data based on 400 seeds, MRL= Mean root length, MSL= Mean shoot length, SE= Standard error; Mean separation with in columns by Duncan's Multiple range test (DMRT). Values followed by different alphabets are significantly different at $P < 0.05$.

Table 3. Effect of combinations of insecticide and fungicides on quality parameters of okra

Treatment	Concentration of chemicals	Dry root weight (g)	Dry shoot weight (g)	No. of leaves/ plant	No. of fruit/ plant	Mean length of the fruit(cm)	Girth of the fruit (cm)	Wt. of the fruit	No. of seeds/ fruit	1000 seed weight (g)	Ascorbic acid (mg)
Goucho+Anucop	0.2+0.06	0.09±0.0^{c} (018±0.3)^{d}	0.2±0.0^{c} (1.0±0.2)^{c}	4.0±0.0^{b} (4.0±0.0)^{b}	3.0±0.0^{d} (3.0±0.0)^{d}	16.5±0.5^{c} (16.5±05)^{f}	6.0±0.0^{d} (6.0±0.0)^{d}	24.5±0.5^{e} (26.5±0.5)^{f}	50.0±0.0^{g} (50.0±0.0)^{g}	61.0±1.3^{d} (61.0±1.0)^{e}	28.5±0.5^{d} (28.5±0.5)^{e}
Goucho+Bavistin	0.2+0.06	0.1±0.3^{b} (0.25±0.3)^{b}	0.5±0.2^{a} (1.9±0.2)^{a}	4.0±0.0^{b} (4.0±0.0)^{b}	4.0±0.0^{c} (4.5±0.5)^{b}	20.5±0.5^{a} (21.0±0.0)^{b}	9.5±0.5^{a} (10.0±0.0)^{b}	34.5±0.5^{b} (35.5±0.5)^{b}	75.5±0.5^{b} (78.5±0.5)^{b}	75.0±0.0^{a} (75.0±0.0)^{b}	35.5±0.5^{a} (355±0.5)^{b}
Goucho+Captan	0.2+0.10	0.2±0.2^{a} (0.19±0.3)^{d}	0.5±0.0^{a} (1.5±0.2)^{b}	5.0±0.0^{a} (5.5±0.5)^{a}	5.5±0.5^{a} (5.5±0.5)^{a}	17.5±0.5^{b} (18.0±0.0)^{f}	6.5±0.53^{d} (7.0±0.0)^{d}	30.0±0.1^{c} (30.0±0.0)^{d}	59.0±1.0^{e} (60.0±0.0)^{e}	60.5±0.5^{d} (62.0±0.0)^{e}	30.0±0.0^{c} (30.0±0.0)^{d}
Gcucho+Dithane M-45	0.2+0.06	0.01±0.2^{e} (0.16±0.3)^{d}	0.3±0.2^{c} (1.1±0.3)^{c}	5.0±0.0^{a} (4.5±0.5)^{b}	4.0±0.0^{c} (4.0±0.0)^{c}	15.5±0.5^{d} (16.0±0.0)^{f}	5.5±0.5 (6.0±0.0)^{e}	24.5±0.5^{e} (25.0±0.0)^{f}	42.5±0.55^{h} (45.0±0.0)^{h}	0.0±0.0^{f} (50.0±0.0)^{f}	23.0±0.0^{e} (23.0±0.0)^{f}
Goucho+Vitavax	0.2+0.10	0.08±0.1^{d} (0.15±0.0)^{d}	0.2±0.0^{b} (0.6±0.0)^{e}	4.0±0.0^{b} (4.5±0.5)^{b}	3.0±0.0^{d} (3.5±0.5)^{d}	13.0±0.0^{e} (14.0±0.0)^{g}	4.5±0.5 (5.0±0.0)^{e}	22.5±0.5^{f} (23.0±0.0)^{h}	38.0±0.0^{i} (40.0±0.0)^{j}	45.5±0.5^{g} (45.5±0.5)^{g}	21.0±0.0^{f} (21.5±0.5)^{g}
Goucho+Bavistin+ Captan	0.2+0.6+0.10	0.1±0.0^{b} (0.5±0.0)^{a}	0.6±0.0^{a} (0.9±0.0)^{d}	5.0±0.0^{a} (5.0±0.0)^{a}	5.0±0.0^{b} (5.0±0.0)^{a}	20.0±0.0^{a} (22.0±0.0)^{a}	10.5±0.5^{a} (11.0±0.0)^{a}	38.0±0.0^{a} (40.0±0.2)^{a}	80.0±0.0^{a} (82.0±0.0)^{a}	74.0±1.0^{a} (78.0±0.0)^{a}	35.5±0.5^{a} (38.0±0.0)^{a}
Goucho+Captan+ Dithane M-45	0.2+0.10+0.06	0.09±0.0^{c} (0.2±0.0)^{b}	0.2±0.0^{b} (1.9±0.0)^{a}	4.0±0.0^{b} (4.0±0.0)^{c}	3.0±0.0^{d} (3.0±0.0)^{d}	18.0±0.0^{b} (19.0±0.0)^{d}	7.0±0.0^{c} (8.0±0.0)^{d}	30.0±0.0^{c} (31.0±0.0)^{d}	62.5±0.5^{d} (64.5±0.5)^{d}	65.0±0.0^{c} (67.0±1.0)^{d}	30.0±0.0^{c} (30.0±0.0)^{d}
Goucho+Anucop+ Captan	0.2+0.10+0.10	0.09±0.0^{c} (0.2±0.0)^{b}	0.2±0.0^{b} (1.8±0.2)^{a}	4.0±0.0^{b} (4.0±0.0)^{c}	4.0±0.0^{c} (4.0±0.0)^{c}	16.5±0.5^{c} (18.0±0.0)^{e}	6.8±0.2^{d} (7.8±0.2)^{e}	27.5±0.5^{d} (28.0±1.0)^{e}	55.5±0.5^{f} (57.5±0.5)^{f}	55.0±0.0^{e} (55.0±0.0)^{f}	35.0±0.0^{a} (35.7±0.2)^{b}
Goucho+Vitavax+ Anucop	0.2+0.06+0.10	0.1±0.3^{b} (0.2±0.0)^{c}	0.6±0.0^{a} (1.6±0.0)^{b}	4.0±0.0^{b} (4.0±0.0)^{c}	4.0±0.0^{c} (4.0±0.0)^{c}	18.0±0.0^{b} (20.0±0.0)^{c}	8.0±0.0^{b} (9.0±0.0)^{c}	31.0±0.0^{c} (33.0±0.0)^{c}	65.5±0.5^{c} (70.0±0.0)^{c}	70.0±0.0^{b} (70.5±0.5)^{c}	32.0±0.0^{b} (32.5±0.5)^{c}
Control	-	0.07±0.0^{d} (0.14±0.3)^{d}	0.1±0.0^{c} (0.5±0.2)^{f}	3.0±0.0^{c} (3.0±0.0)^{d}	3.0±0.0^{d} (3.0±0.0)^{d}	12.0±0.0^{f} (13.0±0.0)^{h}	4.0±0.0^{f} (4.5±0.0)^{f}	20.0±0.0^{g} (20.5±0.5)^{l}	5.5±0.5^{j} (37.0±1.0)^{j}	42.0±0.0^{h} (42.5±0.5)^{l}	20.0±0.0^{g} (20.0±0.0)^{h}

Data based on 400 seeds, Data in parenthesis refers to the field observation, Data other than parenthesis indicate the values for greenhouse studies; Mean separation with in columns by Duncan's Multiple range test (DMRT). Values **followed** by different alphabets are significantly different at $P \leq 0.05$.

Table 4. Combined effects of insecticides and fungicides on seed mycoflora of okra seeds procured from treated plants

Fungi	Control	% occurrence of fungi in okra seeds obtained from plants treated with insecticides and fungicides in combination							
		G+V	G+B	G+C	G+D	G+A	G+B+C	G+C+D	G+A+C	G+V+A
Actinomycete species	31(30)	1(1)	0(0)	2(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Alternaria alternata	30(25)	4(3)	0(0)	0(0)	0(0)	2(2)	0(0)	0(0)	0(0)	0(0)
Aspergillus flavus	5(5)	4(3)	0(0)	0(0)	4(2)	4(3)	0(0)	0(0)	0(0)	0(0)
Aspergillus fumigatus	4(4)	4(4)	0(0)	0(0)	2(2)	2(2)	0(0)	0(0)	0(0)	0(0)
Aspergillus columnaris	5(4)	2(1)	0(0)	2(1)	2(2)	2(2)	0(0)	0(0)	0(0)	0(0)
Aspergillus niger	5(5)	0(0)	0(0)	2(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Botryodiplodia theobromae	5(4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Chaetomium globosum	3(2)	2(2)	0(0)	0(0)	0(0)	1(1)	0(0)	0(0)	0(0)	0(0)
Colletotrichum dematium	2(2)	2(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Fusarium moniliforme	70(65)	2(1)	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Fusarium solani	22(20)	1(1)	0(0)	0(0)	2(0)	2(2)	0(0)	0(0)	0(0)	0(0)
Macrophomina phaseolina	71(68)	0(0)	0(0)	0(0)	1(0)	2(2)	0(0)	0(0)	0(0)	0(0)
Rhizoctonia solani	2(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Rhizopus stolonifer	10(8)	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Trichothecium roseum	1(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Data based on 400 seeds. Data presented in parenthesis Control = seeds obtained from plants not treated with any refer to field evaluation insecticide or fungicide.

Current findings explain the superiority of combination of insecticide with fungicides such as Goucho + Bavistin, Goucho + Bavistin + Captan, Goucho + Captan + Dithane, Goucho + Anucop + Captan and Gocho + Vitavax + Anucop compared to Goucho + Dithane, Goucho + Anucop, Goucho + Captan, Goucho + Captan and Goucho + Vitavax with respect to seed mycoflora, seed germination, vigour index, even under field condition. Therefore, seed treatment with insecticide along with different combinations of fungicides reduces seedborne fungi and thus improves the seed germination, seedling vigour, quantity and quality of okra seeds.

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