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Full Research Article

Strategy for the Management of Bacterial Spot of Bottle Gourd Caused by *Xanthomonas cucurbitae* under Low Hill Conditions of Himachal Pradesh

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Abstract

To manage the bacterial spot of bottle gourd caused by *Xanthomonas cucurbitae,* experiments were conducted at College of Horticulture and Forestry (then Institute of Biotechnology and Environmental Science), Neri Hamirpur (HP) during 2010, 2011 and 2012 cropping seasons. Five chemicals viz., zineb, Bodeaux mixture, mancozeb, copper oxychloride and streptocycline were evaluated as foliar sprays on bottle gourd grown under field conditions during 2010. Minimum disease severity (28.33%) and maximum disease control (68.45%) was recorded in Streptocycline treated plants followed by copper oxychloride. During 2011, naturally infected seed was treated with streptocycline (100 ppm) and copper oxychloride (3000 ppm) alone and in combination with each other for 1.0, 2.0 and 3.0 hours. Seeds treated for three hours in combination of streptocycline and copper oxychloride proved best in terms of seed germination and exclusion of bactria. During 2011, effective seed treatment and foliar sprays were evaluated alone or in combinations under natural conditions on six genotypes of bottle gourd. Mean disease severity in all genotypes of bottle gourd ranged between 33.75 to 35.83%, while, a combination of seed treatment with streptocycline (0.01%)+copper oxychloride (0.3%) for 3.0 h and foliar spray with the same combination resulted in significantly minimum disease severity (20.56%) and maximum disease control (76.34%). During 2012 crop season, spray interval of two effective treatments was standardized in combination with removal of diseased plant parts. Above seed treatment followed by the spray of streptocycline (0.01%)+copper oxychloride (0.3%) at 10 days interval alongwith the removal of diseased plant parts proved most effective in managing (78.35% disease control) the disease (20.17%) in comparison to untreated control.

1. Introduction

Bottle gourd (*Lagenaria siceraria* syn. L. *vulgaris*) belonging to family Cucurbitaceae is one of the important vegetable crops of tropical and sub-tropical areas in India. Its fruits are cooked as vegetable and are also used for preparing sweetmeats, rayata and pickles etc. Additionally, it is also used as a medicinally important crop as the fruits are good source of vitamin B, have cooling effect, prevent constipation and are easily digestible (Yawalkar, 1992). In Himachal Pradesh, the crop is grown as a commercial vegetable in subtropical zone of the state. In recent past the crop has been reported to be attacked by a bacterial spot disease caused by *Xanthomonas cucurbitae* (Jarial et al., 2011). The disease has become so severe in the region that the farmers have reduced or even stopped the cultivation of the crop. The yield losses as high as 70% have been reported due to this disease (Jarial et al., 2011). Earlier, the disease was reported on cucumber from Bihar in India (Sinha, 1989) with preliminary investigations on the management of the disease but, after that no work has been done on the management aspect of the disease in India. The pathogen is emerging as an important pest of different cucurbits world over (Kushima et al., 1994; Vlasov, 2005; Pruvost et al., 2008; Lamichhane et al., 2010; Babadoost and Ravanlou, 2012; Dutta et al., 2013; Salamanca, 2014; Trueman et al., 2014) and has been reported to cause significant losses. According to Larazev (2009) yield losses may reach more than 20% in highly susceptible cultivars, and the disease severity sometimes reaches 50-60% at fruit storage in different cucurbits. Babadoost and Ravanlou (2012) has reported yield losses varying between 3 to 90% in case of pumpkin fields due to this disease in Illinois. The pathogen is internally seed borne (Vincent-Sealy and Brathwaite, 1982) and requires its treatment in the seed prior to sowing. The disease management strategy should include integration of all effective methods in the area (Babadoost and Zitter, 2009). Keeping in view the importance of disease in the region, present investigations were undertaken with an objective to develop an effective seed treatment method and a suitable strategy for the management of disease.

2. Materials and Methods

Experiments were conducted as randomized block design in the experimental farm of the College of Horticulture and Forestry (then Institute of Biotechnology and Environmental Science) Neri, Hamirpur during the cropping seasons of 2010, 2011 and 2012. During 2010 cropping season, seeds of bottle gourd cv. Pusa Summer Prolific Long were sown during the last week of May in poly bags. Germinated plants were transplanted in the field after 15-20 days. Proper staking was given to each vine for its climbing and spread. Normal package of practices were followed to raise the crop. As the disease started appearing in the field, five effective chemicals selected after *in vitro* studies (Jarial et al., 2011) were applied as foliar sprays on the vines and the sprays were repeated at 10 days interval. In all, four sprays were given during the whole cropping period and each treatment was replicated four times. Vines left unsprayed served as control. Data were recorded in terms of disease severity based on the scale given by Jarial et al. (2011) and presented as disease index (%) calculated as per the formula of Mc Kinney (1923). Disease control (%) was further calculated as follows:

Disease control (%)=C-T/C×100

C: is the disease severity (%) in untreated control plot

T: is the disease severity (%) in fungicide treated plot

Table 1: Efficacy of different foliar sprays against bacterial spot of bottle gourd during 2010 crop season

Treatment	Disease severity (%)	Disease control (%)
Zineb (0.25%)	48.33 (44.04)	46.25
Bordeaux Mixture (4:4:50)	43.33 (41.16)	51.80
Mancozeb (0.25)	48.33 (44.04)	46.25
Copper oxychloride 0.3%)	36.67 (37.26)	59.20
Streptocycline (0.01%)	28.33 (32.14)	68.45
Untreated control	90.00 (71.95)	
SE	3.04	
CD (p=0.05)	5.43	

Figures in parentheses are arc sine transformed values

In another experiment, naturally infected seeds were collected from the diseased vines during 2010 and were brought to laboratory. These infected seeds were dip treated with chemicals found effective in field experiment i.e., streptocycline (100 ppm) and copper oxychloride (3000 ppm) alone and in combination with each other for 1.0, 2.0 and 3.0 h. Data were recorded in terms of seed germination (%). Further, the isolations were taken from germinated seeds on nutrient agar medium to see the presence of bacterium in the seeds after the treatment and percentage of seeds carrying bacterium was calculated out of total number of seeds treated.

During 2011 crop season, effective seed treatment and foliar sprays from previous experiments were tested either alone or in combinations under natural epiphytotic conditions on six varieties/ lines/ hybrids of bottle gourd. First spray was started with the appearance of symptoms on the leaves and in all, three foliar sprays were given at 15 days interval. Data were recorded in terms of disease severity presented as disease index (%) and disease control (%) was further calculated as above.

Two best treatments from 2011 field experiment were selected further during 2012 crop season to standardize the spray interval and to see the effect of removal of diseased plant parts with these treatments on disease level. The treated seed of bottle gourd cv Pusa Summer Prolific Long was sown under natural epiphytotic conditions as mentioned above and spray was started at the first appearance of symptoms on the leaves. In different treatments, sprays were repeated at 10 and 15 days interval and the diseased plant parts were removed. Untreated seeds sown and their vines served as control. The data on disease severity and fruit yield were recorded and per cent disease control was further calculated as mentioned above. Finally, a suitable strategy was devised for the management of disease.

3. Results and Discussion

During 2010, out of different chemicals tested as foliar sprays (Table 1), streptocycline proved statistically most effective in reducing disease severity (28.33%) and increasing disease control (68.45%) followed by copper oxychloride. However, in general all the chemicals tested were statistically better than untreated control. Plants sprayed with mancozeb and zineb exhibited maximum disease severity (48.33%) next to untreated check and minimum disease control (46.25%). These results are in conformity with the studies of Sinha (1989) who also reported foliar sprays of streptocycline and copper oxychloride to be effective and mancozeb to be less effective against the disease. From these studies streptocycline and copper oxychloride were selected for further experiments.

Seeds collected from naturally infected gourds were collected and subjected to various seed treatments with streptocycline and copper oxychloride alone or in combination with each other (Table 2). It is evident from the Table that the bacterium could be isolated in 0 to 65% seeds treated with both the chemicals either individually or in combination in comparison to untreated control where the bacterium was isolated from 90% seeds. The bacterium was easily isolated from higher percentage of seeds (35 to 65%) dip treated up to 2 h duration irrespective of the chemical tested and was eradicated totally after 3 h of dip treatment with a combination of both chemicals, though it could be isolated from 10 to 15% seeds treated for 3h with these chemicals individually. As far as seed germination was concerned, there was negligible difference in seed germination percentage of a dip treatment for same duration in different chemicals. However, it ranged between 87 to 98% in different seed treatment as compared to 66% germination in untreated control seeds. So, finally a seed treatment with a combination of streptocycline (100 ppm) plus copper oxychloride (3000 ppm) for 3.0 h was selected for further management trials. According to Moffett and Wood (1979), seed transmission of the bacterium was eliminated by soaking the infested seed in a 1:20 dilution of commercial hydrochloric acid containing 1% spreader-sticker for 60 minute while, hot water treatment at 54 to 56 °C for 30 minutes and a 1% sodium hypochlorite+1% spreader-sticker treatment for 40 min greatly reduced the level of seed transmission but did not eliminate it. Seed treatments (dry heat, hot water, sodium hypochlorite, etc.) can reduce the bacterial numbers in the seed, but will not eliminate it completely (Salamanca, 2014). However, during present studies a seed treatment in combination of streptocycline and copper oxychloride for 3 h could eliminate the bacterium completely but, there are no reports of seed treatment with streptocycline or copper oxychloride in the literature and thus these results cannot be compared with.

Table 2: Seed germination and isolation of pathogenic bacterium from the bottle gourd seeds dip treated with chemicals

Seed dip treatment	Duration (h)	Seed germination (%)	Bacterium isolated in seeds (%)
Streptocycline (100 ppm)	1.0	98.33	50
Streptocycline (100 ppm)	2.0	93.33	35
Streptocycline (100 ppm)	3.0	87.67	10
Copper oxychloride (3000 ppm)	1.0	98.33	65
Copper oxychloride (3000 ppm)	2.0	92.67	40
Copper oxychloride (3000 ppm)	3.0	88.00	15
Streptocycline (100 ppm)+Copper oxychloride (3000 ppm)	1.0	98.67	35
Streptocycline (100 ppm)+Copper oxychloride (3000 ppm)	2.0	92.67	20
Streptocycline (100 ppm)+Copper oxychloride (3000 ppm)	3.0	87.67	0
Untreated control		66.67	90

During 2011 crop season, effective seed treatment and foliar sprays were evaluated individually or in combination with each other under field conditions and the results obtained have been presented in (Table 3). A perusal of the data reveals that the disease was prevalent in all the genotypes of bottle gourd tested and there were non significant differences in the disease levels among all the genotypes ranging from 33.75 to 35.83% irrespective of different seed treatments and / or foliar spray applications. However, as far as different treatments were concerned, it was found that mean disease severity was statistically minimum (20.56%) in the plants raised after seed treatment with above mentioned combination followed by four foliar sprays of same combination of chemicals exhibiting maximum disease control (76.34%) but the treatment was statistically at par with seed treatment plus alternate sprays of streptocycline and copper oxychloride exhibiting 20.83% disease severity and 76.03% disease control. Seed treatment plus foliar sprays of streptocycline proved second best (71.23 % disease control) followed by only foliar sprays with a combination of streptocycline and copper oxychloride (68.36 % disease control). Foliar sprays of copper oxychloride alone proved least effective exhibiting 38.06% disease severity and about 56% disease control in comparison to 89.94% disease severity in untreated control plots.

Finally in 2012, best two treatments from previous year studies were further evaluated where foliar sprays were given at 10 and 15 days interval along with or without the removal of diseased plant parts. It was found that a combination of above seed treatment with four foliar sprays of same combination at 10 days interval along with the removal of diseased plant parts proved most effective in managing the disease (20.17%) upto 78.35% followed by a combination of same seed treatment and alternate foliar sprays of these two chemicals at ten days interval combined with the removal of diseased plant parts exhibiting 22.67% disease severity and 75.67% disease control as compared to untreated control. It was observed that same combination of chemicals exhibited significant differences in disease levels when sprayed at 10 and 15 days interval.

Table 3: Efficacy of different chemical treatments against bacterial spot in various genotypes of bottle gourd during 2011 crop season

Treatments	Disease severity (%) in variety / hybrid						Overall mean	Disease control (%)
	CBH- 7	CBH-8	CBH-9	PM	PSPL	Local sel		
T_1	31.67 (34.23)	31.67 (34.15)	30.00 (33.16)	33.33 (35.25)	30.00 (33.16)	31.67 (34.23)	31.39 (34.39)^c	63.88
T_2	31.67 (34.23)	31.67 (34.23)	31.67 (34.23)	30.00 (33.16)	31.67 (34.23)	30.00 (33.16)	31.11 (33.87)^c	64.20
T_3	38.33 (38.25)	35.00 (36.24)	38.33 (38.25)	40.00 (39.21)	40.00 (39.21)	36.67 (37.26)	38.06 (38.07)^d	56.21
T_4	28.33 (32.14)	21.67 (27.71)	28.33 (32.14)	23.33 (28.86)	26.67 (31.07)	26.67 (31.07)	25.83 (30.50)^b	70.28
T_5	31.67 (34.23)	25.00 (29.93)	28.33 (32.02)	25.00 (29.80)	28.33 (32.14)	26.67 (30.95)	27.50 (31.57)^b	68.36
T_1+T_2	28.33 (32.09)	26.67 (31.07)	23.33 (28.78)	23.33 (28.86)	25.00 (29.93)	23.33 (28.86)	25.00 (29.93)^b	71.23
T_1+T_3	35.00 (36.27)	30.00 (33.16)	30.00 (33.16)	33.33 (35.25)	31.67 (34.23)	30.00 (33.16)	31.67 (34.21)^c	63.56
T_1+T_4	23.33 (28.86)	16.67 (24.05)	21.67 (27.71)	20.00 (26.57)	20.00 (26.45)	21.67 (27.71)	20.56 (26.89)^a	76.34
T_1+T_5	20.00 (26.45)	20.00 (26.45)	21.67 (27.71)	20.00 (26.45)	20.00 (26.45)	23.33 (28.86)	20.83 (27.06)^a	76.03
Control	90.00 (71.95)	86.67 (69.24)	88.33 (70.12)	86.67 (68.67)	86.67 (68.67)	83.33 (66.15)	89.94 (69.13)^f	
Overall mean	35.83 (36.85)^b	33.75 25.39)^a	34.58 (35.97)^a	34.58 (35.87)^a	35.28 (36.32)^a	34.58 (35.89)^a		

Figures in parentheses are arc sine transformed values

SE = Variety 0.74 Treatment 1.08 Variety×Treatment 2.74

CD (p=0.05) = Variety 1.31 Treatment 1.85 Variety×Treatment 4.54

Where, T_1: Seed Treatment with Streptocycline+Copper Oxychloride for 3.0 h; T_2: Foliar Spray with Streptocycline; T_3: Foliar Spray with Copper Oxychloride ; T_4: Foliar Spray with Streptocycline+Copper Oxychloride; T_5: Alternate Foliar Spray with Streptocycline and Copper Oxychloride; PM: Pusa Manjari; PSPL: Pusa Summer Prolific Long

Additionally, the removal of diseased plant parts further decreased the diseased levels significantly indicating a specific role of diseased plant parts in spread of disease. Fruit yield was maximum (14.350 kg 4 m^{-2}) in the vines raised from above mentioned seed treatment followed by four foliar sprays of streptocycline plus copper oxychloride at 10 days interval along with removal of diseased plant parts which was statistically at par with the yield (14.000 kg 4 m^{-2}) in plots treated with same seed treatment followed by four foliar sprays of above two chemicals alternatively along with removal of diseased plant parts (Table 4). However, there was a negative correlation between disease severity and fruit yield being more in vines having lesser disease and *vice-versa*. The yield was recorded to be significantly minimum (3.5 kg 4 m^{-2}) in untreated control plots having disease severity of 93.17% exhibiting the serious impact of disease on fruit yield. These studies clearly revealed the importance of spray intervals and removal of diseased plant parts in the disease management. There are no reports in the literature on such type of studies in this disease hence, these results cannot be compared with. However, according to Salamanca (2014), frequent foliar application of preventative sprays can help decrease the bacterial population in the field to some extent. Sinha (1989) reported that 8 foliar sprays of chemicals like plantomycin, paushamycin, streptocycline, Ceresanwet (phenyl mercury acetate), Blitox-50 (copper oxychloride) and captan were quite effective in managing the disease. Although, preventive application of copper can reduce the number or plants infected and the severity of disease development in the field, but has limited efficacy on years with high rainfall, but preventative application of copper formulations is considered more efficacious compared with sprays after the symptoms have developed (Salamanca, 2014). Application of copper compounds during early formation and

Table 4: Effect of different management practices and spray intervals on bacterial spot and yield of bottle gourd during 2012 crop season

Treatment	Disease severity (%)	Disease control (%)	Yield (kg 4 m^{-2})
T_1+T_4 at 10 days interval (F_1)	25.11 (30.08)bc	73.05	13.675a
F_1+Removal of diseased plant parts	20.17 (26.68)a	78.35	14.350a
T_1+T_4 at 15 days interval (F_2)	32.00 (35.25)e	65.65	12.000bc
F_2+Removal of diseased plant parts	30.50 (33.26)d	67.26	12.050bc
T_1+T_5 at 10 days interval (F_3)	26.08 (30.72)c	72.01	12.900ab
F_3+Removal of diseased plant parts	22.67 (28.45)b	75.67	14.000a
T_1+T_5 at 15 days interval (F_4)	35.00 (36.28)e	62.43	11.000c
F_4+Removal of diseased plant parts	32.42 (35.84)e	65.20	11.890bc
Untreated Control	93.17 (75.86)f		3.500d
SE	0.99		0.840
CD (p=0.05)	1.73		1.457

expansion of fruit may result in substantial fewer symptomatic fruits (Anonymous, 2012).

4. Conclusion

Integration of seed treatment, foliar sprays and cultural methods can help reducing the disease levels. So a management strategy comprising of a seed treatment with streptocycline (0.01%) plus copper oxychloride (0.3%) and four foliar sprays of same combination at ten days interval along with the removal of diseased plant parts regularly during the cropping season is a useful strategy against bacterial spot of bottle gourd and can be recommended for its management.

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