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HORTICULTURE

Effect of Agro-chemicals on Severity of Bacterial Blight and Fruit Quality in Pomegranate

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Abstract

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is a wide spread disease affecting pomegranate production and quality of fruit in Punjab. *In-vitro* evaluation of agrochemicals indicated that blitox at 3000 ppm followed by kocide 2500 ppm successfully managed the disease. A field experiment was also conducted for its management at New orchard, Punjab Agricultural University, Ludhiana during, 2013. Three sprays of the agro-chemicals were done at 15 days interval starting from end June to end July on Mridula variety of pomegranate. Among the various treatments, blitox (0.3%) + streptocycline (250 ppm) proved most effective in reducing per cent disease index, per cent fruit cracking and providing maximum disease control. Quality parameters viz., TSS, acidity, weight, pulp colour, juice weight etc. were also studied. Maximum TSS, fruit weight, juice weight, pulp weight, 100 grain weight and total grain weight were observed in blitox (0.3%) + streptocycline (250 ppm) followed by kocide (0.25%) + streptocycline (250 ppm) sprayed fruits.

Highlights

- Maximum mean inhibition zone was achieved with fungicides rather than antibiotics.
- Blitox (0.3%) + streptocycline (250 ppm) proved significantly superior to other agro-chemicals in reducing per cent disease index.
- Quality analysis of the fruits revealed that maximum TSS, fruit weight, juice weight, pulp weight, 100 grain weight and total grain weight were in blitox (0.3%) + streptocycline (250 ppm).

Keywords: Agro-chemicals, bacterial blight, management, pomegranate, quality analysis, *Xanthomonas axonopodis* pv. *punicae*

Pomegranate is an ancient fruit, native of Iran, where it was first cultivated in 2000 BC and then spread to Mediterranean countries including Egypt, Iran Afghanistan, Arabia and Baluchistan (Evreinoff 1949). In India, pomegranate was grown in an area of 112.74 thousand hectare with production of 741.08 thousand MT in 2012-13 (Anonymous 2013). India ranks second with an annual export of 33,415 tons after Iran (67,000 tons) (Jadhav and Sharma 2009). Pomegranate fruit has wider adaptability as it grows very well on moderately alkaline as well as slightly acidic soils. It has wide consumer preferences for its attractive, juicy, sweet-acidic and refreshing arils. Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. Glucose (5.46%) and fructose (6.14%) are the main sugars with no sucrose in fruits. It is rich in vitamin-C and citric acid is the most predominant organic acid in pomegranate (Malhotra *et al.,* 1983). Pomegranate cultivation is associated with many constraints. Among the biotic constraints, bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is a wide spread disease affecting its successful production and every year results into 50-100 per cent economic losses depending upon disease severity.

Material and Methods

In vitro evaluation of agro-chemicals

Three contact fungicides viz., Blitox (50 WP), Kocide (77 WP) and Bordeaux Mixture (2:2:250) and two antibiotics viz., Streptocycline and Bactrinashak (0.05%) at five different cconcentrations were evaluated for their efficacy against the growth of *X. axonopodis* pv. *punicae* by inhibition zone assay method. The bacterium was multiplied by inoculating the culture into the 20 ml of nutrient broth taken in 'Erleyenmayers' flask. The inoculated flasks were incubated at 28°C for 72 hours.

The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petriplates and plates were allowed to solidify. The chemical solutions were prepared at different concentrations. The filter paper discs (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective chemical solution for 5 minutes and transferred onto the surface of the seeded medium in Petriplates. The inoculated plates were kept in the refrigerator at 5°C for 4 hours to allow the diffusion of chemical into the medium. Then plates were incubated at 28°C for 72 hours and observed for the production of inhibition zone around the filter paper discs. The data were statistically analyzed using completely randomized block design.

In vivo evaluation of chemicals

The Field experiment was conducted on three years old plants of Mridula variety at New orchard, Punjab Agricultural University, Ludhiana during 2013. Pruning of diseased twigs was done during February. The agro-chemicals viz. blitox 50 WP (copper oxychloride, 0.3%), blitox 50 WP (copper oxychloride, 0.3%) + streptocycline [streptomycin sulphate (90%) + tetracycline hydroxide (10%), 250 ppm], kocide 77 WP (copper hydroxide, 0.25%), kocide 77 WP (copper hydroxide, 0.25%) + streptocycline [streptomycin sulphate (90%) + tetracycline hydroxide (10%), 250 ppm], bactrinashak (2-Bromo-2-nitro propane-1,3-diol, 0.05%) and Bordeaux mixture (2:2:250) were sprayed. Three sprays were done at 15 days interval starting from end June to end July. The experiment was laid out in a Randomized Block Design. Each treatment was replicated thrice by keeping single plant per replication along with control.

Periodical observations were recorded in terms of per cent disease index on leaves and fruits after each spray at 15 days interval using 0-4 scale (Chester 1950) where, 0= no spot visible on the leaves, 1= one-fourth of the leaf area spotted, 2= half of the leaf area spotted, 3= three-fourth of the leaf area spotted and 4= more than three-fourth of the leaf area spotted. The data on fruit yield was also recorded at the time of harvest.

Quality analysis of the fruits collected from treated trees was done in Post-harvest laboratory, Department of Fruit Science, PAU, Ludhiana. The following parameters were studied for quality analysis of the fruits:

1. Fruit weight, pulp weight, 100 grain weight, total grain weight and juice weight was measured with weighing balance.
2. Length, breadth was measured with measuring scale.
3. **Total Soluble Salts (TSS):** Total soluble solids (TSS) content were determined from the fruit juice at room temperature with the help of rafractrometer (0-32 °Brix) and expressed in per cent. The readings were corrected with the help of temperature correction chart at 20°C temperature
4. **Acidity:** Acidity was determined by titrating 2 ml of strained juice of fruits against 0.1 N NaOH solution using phenolphthalein as an indicator. The appearance of light pink colour marked the end point of titration.
5. **Palatability Rate:** The organoleptic rating of the fruit was conducted by a panel of five judges. The characters viz., appearance of fruits, taste, texture and eating quality were taken into consideration by the panel for its evaluation. A nine point Hedonic scale described by Amerine *et al.* (1965) was used for its inference, as given below:

Score	**Acceptability**
9	Extremely desirable
8	Very much desirable
7	Moderately desirable
6	Slightly desirable
5	Neither desirable and nor undesirable

4	Slightly undesirable
3	Moderately undesirable
2	Very much undesirable
1	Extremely undesirable

6. **pH:** It was measured with pH meter.
7. **Pulp Colour:** The colour of fruits was measured with colour difference meter (Colour Flex, Hunter Lab, USA) and expressed as a, b hunter colour values. The instrument measured value viz., l, a and b.

Results and Discussion

***In vitro* evaluation of agro chemicals:** An experiment (Table 1) was carried out to evaluate commercially available chemicals to find out their effectiveness against the growth of *Xanthomonas axonopodis* pv *punicae* under *in vitro* condition. It is clearly indicated from table 1 that among contact fungicides and antibiotics, maximum mean inhibition zone (cm) was achieved with fungicides rather than antibiotics. Blitox at concentration of 3000 ppm found effecvtive followed by kocide at concentraion of 2500 ppm. Streptocycline found effective at concentration >200 but less than 500 ppm. Bactrinashak proved least effective in inhibiting the growth of bacteria. Growth was also inhibited by Bordeaux mixture at any of the tested concentrations. When these fugicides were tested

Table 1: *In vitro* evaluation of different agro-chemicals against bacterial blight of pomegranate

Sl. No.	Chemical Name	Concentration (ppm)	Mean diameter of inhibition zone (cm)
1	Blitox	1000	2.75 (1.93)
		1500	2.39 (1.84)
		2000	2.23 (1.79)
		2500	1.68 (1.63)
		3000	1.58 (1.60)
	CD(P=0.05)		0.0355
2	Kocide	1000	3.02 (2.00)
		1500	2.61 (1.90)
		2000	2.41 (1.84)
		2500	1.99 (1.72)
		3000	1.63 (1.62)
	CD(P=0.05)		0.0163
3	Streptocycline	20	2.88 (1.96)
		50	2.55 (1.88)
		100	2.25 (1.80)
		200	1.88 (1.69)
		500	1.60 (1.61)
	CD(P=0.05)		0.0621
4	Bordeaux Mixture	1000	2.65 (1.91)
		1500	2.30 (1.81)
		2000	2.00 (1.73)
		2500	1.63 (1.62)
		3000	1.54 (1.59)
	CD(P=0.05)		0.0525
5	Bactrinashak	20	3.11 (2.02)
		50	2.86 (1.96)
		100	2.48 (1.86)
		200	2.19 (1.78)
		500	1.90 (1.70)
	CD(P=0.05)		0.0521

Figure in parentheses are $\sqrt{x+1}$ transformed values with combination of antibiotics (Streptocycline), inhibited maximum bacterial growth.

All chemicals *viz.*, Bordeaux Mixture, Blitox and Kocide were found to be effective but were significantly different from each other. Bactrinashak was less effective and were on par with each other. Between the concentration of each chemicals, efficacy was significant from lower to higher concentration with greater efficacy at higher concentrations. These findings are in agreement with Koizumi (1985), Leite and Mohan (1990) and Graham *et al.* (1992) who recommended Streptocycline and Copperbased bactericides as standard measures for citrus canker control world-wide. Raju (2010) observed that average disease severity recorded was significantly low in all the chemicals treated plots Streptocycline + COC (0.05%+0.25%) treatment indicating greater efficacy of all the bactericides.

***In vivo* evaluation of agro chemicals:** The data presented in Table 2 and Fig. 1 revealed that all the agro-chemicals significantly reduced per cent disease index as compared to control. Blitox (0.3%) + streptocycline (250 ppm) proved significantly superior to other agro-chemicals in reducing per cent disease index. Per cent disease index on the plants sprayed by blitox (0.3%) + streptocycline (250 ppm) was 5.10 and 6.50 on leaves and fruits, respectively, which was considerably lower as compared to control where it was 60.0 and 63.50 per cent on leaves and fruits, respectively.

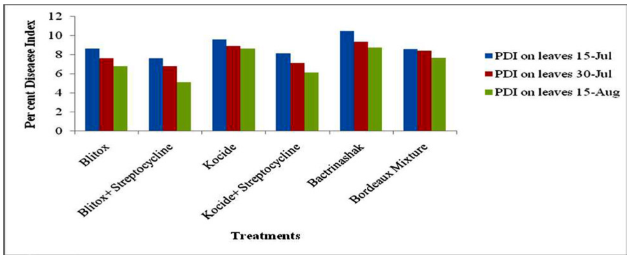

Fig. 1: Field evaluation of different agro-chemicals for management of bacterial blight of pomegranate

Blitox + streptocycline treatment provided maximum disease control (91.50% on leaves and 89.76% on fruits) as compared to other treatments. Kocide (0.25%) + streptocycline (250 ppm) was the next best treatment in minimizing per cent disease index which gave 89.73 and 88.72 per cent disease control on leaves and fruits, respectively. The yield in all the treatments differed significantly as compared to control. Among the treatments maximum (3.85 Kg/plant) yield was obtained in blitox + streptocycline sprayed fruits. Per cent fruit cracking was minimum in the plants sprayed with blitox + streptocycline (2.5%) followed by kocide + streptocycline (3.0%).

Table 2: Field evaluation of different agro-chemicals for management of bacterial blight of pomegranate

Treatment	Concentration	PDI* on leaves			Disease control (%)	PDI* on fruits	Disease control (%)	Yield (Kg/plant)	Fruit cracking (%)
		15-July	30-July	15-Aug					
Blitox	0.3%	8.65 (17.10)	7.61 (16.01)	6.80 (15.11)	88.66	9.93 (18.36)	84.36	3.39 (10.60)	4.0 (11.53)
Blitox+ Streptocycline	0.3%+ 250 ppm	7.62 (16.02)	6.78 (15.09)	5.10 (13.05)	91.50	6.50 (14.77)	89.76	3.85 (11.31)	2.5 (9.09)
Kocide	0.25%	9.59 (18.03)	8.90 (17.36)	8.62 (17.07)	85.63	10.0 (18.43)	84.25	2.98 (9.94)	4.6 (12.38)
Kocide+ Streptocycline	0.25%+ 250 ppm	8.12 (16.56)	7.10 (15.45)	6.16 (14.37)	89.73	7.16 (15.52)	88.72	3.78 (11.21)	3.0 (9.97)
Bactrinashak	0.05%	10.50 (18.90)	9.32 (17.77)	8.75 (17.20)	85.41	10.50 (18.90)	83.46	2.69 (9.43)	5.0 (12.92)
Bordeaux Mixture	2:2:250	8.56 (17.01)	8.39 (16.84)	7.65 (16.05)	87.25	9.75 (18.19)	84.64	3.18 (10.27)	4.4 (12.10)
Control	—	49.00 (44.42)	53.50 (47.00)	60.00 (50.76)		63.50 (52.83)		1.84 (7.80)	8.2 (16.64)
CD (0.05%)		1.83	1.51	2.52		1.22		0.65	0.818

Figure in parentheses are arc sine transformed values

*PDI**- Per cent Disease Index*

The more effectiveness of these agro-chemicals might be due to their more potentiality to reduce inoculum and fresh infection of the pathogen. The results are in accordance with Suriachandraselvan *et al.* (1993), Atulchandra *et al.* (1994), Jadeja *et al.* (2000), Yenjerappa *et al.* (2004), Suryawanshi *et al.* (2009) and Benagi *et al.* (2009). Suriachandraselvan *et al.* (1993) reported that 3 sprays of paushamycin (0.05%) + copper oxychloride (0.2%) at fortnightly intervals was most effective in controlling the bacterial blight on pomegranate caused by *X. campestris* pv. *punicae*. Atulchandra *et al.* (1994) stated that same bacterium could be controlled by spraying of Bordeaux mixture (5:5:50) and other copper fungicides at an interval of 15 days. Yenjerappa *et al.* (2004) noticed the superior efficacy of streptocycline (0.05%) + copper oxychloride (2000 ppm) in checking the bacterial blight menace of pomegranate. Suryawanshi *et al.* (2009) observed that copper oxychloride (0.25%) followed by carbendezim (0.1%) significantly reduced intensity of oily spot disease of pomegranate over untreated control. Benagi *et al.* (2009) revealed that prophylactic spray of streptocycline (0.05%) + copper oxychloride (0.2%) followed by zinc sulphate (0.1%) + boron (0.1%) + magnesium sulphate (0.1%) + calcium sulphate (0.1%) was effective for management of bacterial blight of pomegranate.

Quality analysis of the fruits collected from the treated plants (Table 3, Fig. 2,3 and 4) showed that all the treatments differed significantly as compared to control in respect to weight, length, breadth, TSS, acidity and pulp weight. Among the treatments, maximum (736.75 g) fruit weight was observed in blitox + streptocycline followed by kocide + streptocycline (692.53 g) and Bordeaux mixture (644.35) sprayed fruits. The treatment blitox + streptocycline exhibited maximum length (24.36 cm) and breadth (25.06 cm) followed by kocide + streptocycline having 23.45 cm length and 24.85 cm breadth of the fruit. The maximum (15.6 %) TSS was observed in blitox + streptocycline followed by kocide + streptocycline (15.4 %) and Bordeaux mixture (14.9 %) sprayed fruits. The maximum (61.5g) pulp weight was observed in blitox + streptocycline sprayed fruits. No significant differences were observed among the treatments in respect to acidity. However, minimum (0.25 %) acidity was observed in the fruits which were sprayed with blitox + streptocycline.

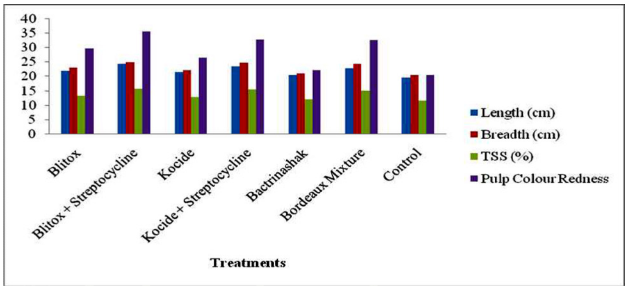

Fig. 2: Quality analysis of pomegranate fruit sprayed with different agro-chemicals

Significant differences were observed in respect to 100 grain weight among all the treatments except kocide and kocide + streptocycline, which were at par. The maximum 100 grain weight was observed in blitox+ streptocycline (29.30g). The total grain

Table 3: Quality analysis of pomegranate fruit sprayed with different agro-chemicals

Treatment	Weight (g)	Length (cm)	Breadth (cm)	TSS (%)	Acidity (%)	Pulp wt (g)	100 grain wt (g)	Total grain wt (g)	Juice wt (g)	PR	pH	Pulp Colour
												Redness
Blitox	572.25	21.96	22.96	13.30	0.29	52.00	18.80	85.45	69.00	8.00	3.50	29.81
Blitox + Streptocycline	736.75	24.36	25.06	15.60	0.25	61.50	29.30	100.40	88.40	8.50	4.05	35.63
Kocide	549.85	21.42	22.23	12.80	0.35	50.00	17.30	80.57	65.50	7.50	3.39	26.59
Kocide + Streptocycline	692.53	23.45	24.85	15.40	0.30	58.00	24.40	98.60	86.50	8.00	3.98	32.92
Bactrinashak	490.03	20.50	21.05	12.00	0.45	43.20	13.80	78.50	52.50	7.00	3.20	22.17
Bordeaux Mixture	644.35	22.75	24.30	14.90	0.29	57.50	19.90	95.50	81.30	8.10	3.75	32.65
Control	430.50	19.65	20.38	11.50	0.56	39.00	10.50	76.28	43.00	6.00	2.90	20.50
CD (p=0.05)	23.26	1.25	0.95	0.98	0.29	1.89	1.14	3.42	2.26	1.05	0.39	1.78

weight was maximum in blitox + streptocycline (100.40 g) followed by kocide + streptocycline (98.60 g) and Bordeaux mixture (95.50) sprayed fruits. The juice weight in blitox + streptocycline sprayed fruits was maximum (88.40g) followed by kocide + streptocycline sprayed fruits (86.5g).

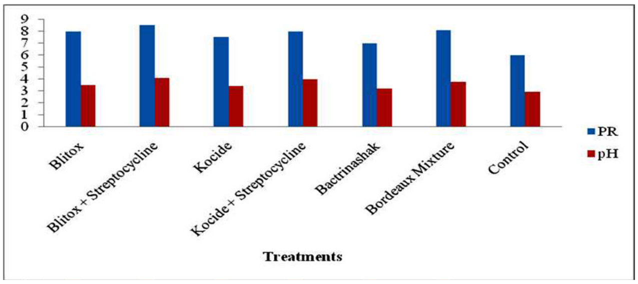

Fig. 3: Quality analysis (PR and pH) of pomegranate fruit sprayed with different agro-chemicals

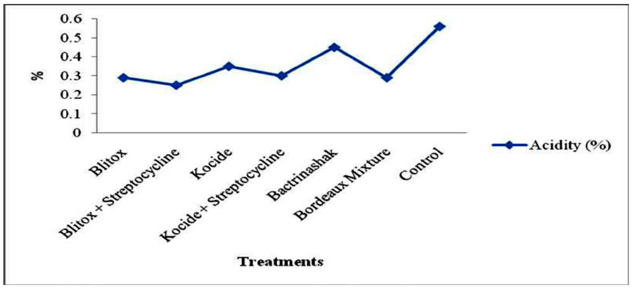

Fig. 4: Quality analysis (Acidity) of pomegranate fruit sprayed with different agro-chemicals

The maximum palatability rate was observed in blitox + streptocycline (8.50) sprayed fruits followed by kocide + streptocycline (8.30). Maximum redness of the pulp was observed in blitox+ streptocycline (35.63) followed by kocide + streptocycline (32.92). The treatments kocide + streptocycline and Bordeaux mixture were at par in respect to pulp colour.

During the present investigation, blitox + streptocycline followed by kocide + streptocycline have shown superiority over other agro-chemicals in reducing per cent disease index on leaves and fruits of pomegranate. Quality analysis of the fruits also revealed that maximum TSS, fruit weight, juice weight, pulp weight, 100 grain weight and total grain weight were in blitox (0.3%) + streptocycline (250 ppm) followed by kocide (0.25%) + streptocycline (250 ppm) sprayed fruits as compared to control.

Similar results were obtained by Haeseler and Petersen 1974 and Valarmathi *et al.* 2013, who reported that the fruit quality of grapevine was improved due to spray of copper hydroxide (kocide 3000). It can be attributed to the fact that the Copper hydroxide effectively controls downy mildew infection in grapevine fruits and thus increases the quality of berries to fetch higher market prices. Ghure and Shinde (1987) observed that there was 3.5, 1.6 and 21.7 per cent reduction in total soluble solids, total sugars and ascorbic acid, respectively, when powdery mildew intensity exceeded 75 per cent. There was also increase in per cent of non reducing sugars (69.7) and acidity (24.3). As a result, fruit taste and market value was reduced. Holb and Schnabel (2005) reported that two or three applications of copper hydroxide to tart cherry during the bloom period for control of brown rot resulted into higher yield as compared to control in a wet year, probably because copper lowered the incidence of brown rot. Leaf discoloration, variously described as bronzing or necrosis, has been associated with the application of copper-based fungicides to tart cherry (McManus *et al.*, 2007 and Gruber *et al.*, 2009). McManus *et al.*, (2007) concluded that applications of copper-based fungicides did not lead to more defoliation assessed at the time of fruit harvest. However, the effects of copper-based fungicides on fruit quality are not well understood because reported results are contradictory and the metrics used to evaluate fruit quantity and quality have differed among studies (Palmer *et al.*, 2003, Lalancette and McFarland 2007 and Jamar *et al.*, 2008).

Conclusion

Blitox (0.3%) + streptocycline (250 ppm) followed by kocide (0.25%) + streptocycline (250 ppm) proved most effective in reducing the per cent disease index, per cent fruit cracking and providing maximum disease control. It was also concluded that TSS, fruit weight, juice weight, 100 grain weight and total fruit weight increased/enhanced in blitox (0.3%) + streptocycline (250 ppm) followed by kocide (0.25%) + streptocycline (250 ppm) sprayed fruits. So, it was revealed that copper salt improved the quality of fruit.

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