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Status and symptomatology of die-back and twig blight diseases of almond (*Prunus amygdalus* Batsch.) in Kashmir valley

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Abstract
Die-back and twig blight are the most important diseases of almond (*Prunus amygdalus* Batsch.) plantation world wide. Survey of almond orchards in Budgam and Pulwama districts revealed the presence of both the diseases with varying degrees of incidence and intensity. On an overall basis die-back recorded the maximum disease incidence and intensity of 22.70 and 10.37 per cent respectively, while incidence of 16.45 per cent and intensity of 7.61 per cent for twig blight. Highest die-back incidence (26.15%) and intensity (11.96%) was recorded in district Budgam while, that of twig blight, 19.85 and 9.12 per cent in incidence and intensity, respectively was also recorded in the same district. Die-back disease was initially characterized by the appearance of light brown lesions on terminal bud scales of current season growth in the first week of June resulting in yellowing and drooping of terminal leaf lets. Later on small light brown lesions appeared on lateral buds which iniciated the chlorosis and necrosis of terminal twig portion. Subsequently progression of chlorosis and necrosis in dawn-ward direction continued throughout the growing season resulting in shriveling and collapsing of the effected twigs. Twig blight initially manifests as minute water soaked lesions near the base of leaf petioles on current seasons terminal growth during 2nd week of June. Elongation of the lesions both in up-ward and down-ward direction along the twig axis resulted in the formation of sunken streaks causing twig girdling. The leaves over the girdled twig wilted and remained clinged unless disturbed through strong breeze or mechanically.

Keywords: Almond, Chlorosis, die-back, management, necrosis, symptoms, twig blight

1. Introduction
Almond (*Prunus amygdalus* Batsch.), belonging to family Rosaceae, is one of the most important nut crop cultivated in temperate regions of the world. The probable origin of almond is believed to be the Mediterranean region (Ladizinsky, 1999) [18]. It was spread by traders in ancient times along the shores of Mediterranean into northern Africa and southern Europe, and more recently distributed to other parts of world, notably California in United States (Zohary and Maria, 2000) [30]. Almond possesses wide spread popularity and is even considered as golden crop of California (Rogers, 1974) [24]. Its cultivation is mainly confined to the countries lying between 36º and 45ºN latitude (Rugini and Monastra, 1991) [25]. World shelled almond production has increased many fold from 1034 metric tonnes in 1995 to 2065 metric tonnes in 2007 (Ahmad and Verma, 2009) [1]. The total world production of almond was estimated 2.51 million metric tonnes in 2010 (FAOSAT, 2013). The main producers of almond (shelled product) include USA (40%), Spain (12.45%), Syria (6.77%), Iran (6.53%), Italy (6.38%), Morocco (4.70%), Algeria (3.39%), Tunisia (2.67%), Greece (2.83%) and Turkey (2.45%) (Ahmad and Verma, 2009) [1]. Almonds are the healthiest and most nutritious nuts of all, considered as a well-balanced cholesterol free food. The 100g of kernel contains 575 calories, fiber (12.2g), vitamin E (26mg), fat (49g), protein (21g), potassium (670mg), magnesium (268mg), phosphorus (484mg), calcium (265mg) and iron (3.5mg) (Ahmad and Verma, 2009) [1]. The medicinal benefits of almond include anti-inflammation, immunity boosting, anti-hepatotoxicity, improved complexion, improved movement of food through colon and prevention of cancer (Davis and Iwahashi, 2001; Puri, 2003) [10, 21]. Almond helps in reducing cardio-vascular diseases by having favourable effect on blood cholesterol levels (Ahmad and Verma, 2009) [1]. In India, almond is mainly grown in the state of Jammu & Kashmir (J&K) and Himachal Pradesh (H.P.) over an area of 23.81 thousand hectares, yielding 11.47 thousand metric tonnes (Kumar, 2010) [17]. However, commercial cultivation of this nut fruit is mainly

confined to the Kashmir Valley. In the Valley, it is mainly grown in district Budgam, Pulwama, Shopian, Gandarbal and other hilly areas occupying an area of 15.93 thousand hectares with a total production of 8.21 thousand MT (Anonymous, 2014) [3]. The productivity of almond in Jammu & Kashmir is 0.73 tonnes per hectare which is more than national productivity of 0.51 tonnes per hectare, but less than the global productivity of 1.15 tonnes per hectare (Anonymous, 2011) [2]. Almond is highly valuable crop and is also used as a filler crop in saffron fields. However, its yield as well as area under cultivation has shown a declining trend during the recent past. The reduction is attributed to several biotic and abiotic factors which include pests and diseases, occurrence of spring frost, poor pollination during cool, cloudy or rainy weather (Qureshi and Dalal, 1985; Connel, 2002) [23, 9]. In addition to this, lack of irrigation facilities, lack of improved varieties and pollinizers are also other important factors which add to declining trend in area and production. Although the almond tree is native to the Mediterranean region, this beautiful tree has adopted to the climate of Kashmir. In spite of favorable environmental conditions for almond cultivation, the tree is attacked by various diseases. During the present few years, almond orchards of the valley have been facing a serious threat due to die-back and twig blight diseases. These disease chiefly attacks the current season twig growth, which ultimately leads to the death of productive wood thereby causing significant reduction in crop yields.

Materials and methods

For assessing the prevalence of die-back and twig blight diseases of almond, an extensive survey was conducted in the months of August-September during the years 2013 and 2014 in two major almond growing districts of Kashmir valley viz., Budgam and Pulwama. Five locations were selected from each district viz., Haproo, Nagam, Nobug, Shelipora and Wahabpora in Budgam and Kralchakh, Gasoo, Kuil, Rhoama and Tahab in Pulwama district. From each location three orchards were randomly selected for survey. In each orchard twenty five trees, representing four corners and centre were randomly selected as representative for each site. Ten current seasons twigs in four cardinal directions in each plant were randomly selected and marked to record the incidence and intensity of diseases, as both the diseases mostly occur on current season growth.

The per cent disease incidence was calculated as per the formula given by James (1974) [15]:

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased twigs}}{\text{Total number of twigs observed}} \times 100$$

The disease intensity was calculated after rating the level of disease on branches and twigs on 0 to 5 scale of Crosse (1957) [8] as per the following key;

Scale used for categorizing die-back and twig blight on almond

Category	Numerical value	Criterion
I	0	No disease
II	1	0.1-10.0% of branch/twig surface area diseased
III	2	10.1-20.0% of branch/twig surface area diseased
IV	3	20.1-30.0% of branch/twig surface area diseased
V	4	30.1-40.0% of branch/twig surface area diseased or one side of branch/twig showing partial gardling
VI	5	More than 40% of branch/twig surface area diseased or complete girdling of the branch/twig. The branch above the girdled portion completely dried

The per cent disease intensity (PDI) was calculated as per the following formula given by FAO (1967) [12]:

$$\text{Per cent disease intensity} = \frac{\Sigma(n \times v)}{N \times S} \times 100$$

Where,
N = Number of branches or twigs in each category
V = Numerical value of each category
N = Total number of branches or twigs examined
S = Maximum numerical value

The detailed studies on the symptom development of die-back and twig blight diseases was undertaken from five seedling origin almond trees grown in a private orchard at Nagam, district Budgam, during 2013. The selected trees were kept unsprayed thought the growing season. From each tree, 10 current season twigs were selected from lower scaffold branches in four cardinal directions. The selected twigs were marked and kept under constant observation for the appearance of disease symptoms. Diseased twigs, showing characteristic symptoms of both the diseases were also collected from different parts of the valley and were critically examined for symptom expression. The disease progress was monitored at weekly intervals from the day of initial symptom appearance with respect to shape, size, colour and subsequent development of die-back and twig blight symptoms. The observations with regards to formation of fruiting bodies on diseased twigs was also recorded. These twigs were also used for the isolation of pathogen (s) and preserved as dry specimen for further investigations.

Results and discussion

Data on prevalence of die-back and twig blight diseases recorded during the survey in two districts of Kashmir valley viz. Budgam and Pulwama during the years 2013 and 2014 are presented in Table 1and 2, respectively. To assess the status of die-back and twig blight diseases of almond an extensive survey was conducted in two major almond growing districts of Kashmir valley viz., Budgam and Pulwama during the year 2013 and 2014. Based on means of two years data the die-back was most prevalent disease (22.70%) incidence and (10.37%) intensity followed by twig blight (16.45%) incidence and (7.61%) intensity. Die-back and twig blight diseases were more or less prevalent in all the almond growing areas of Budgam and Pulwama districts of Kashmir valley during the years 2013 and 2014. However, both the diseases were significantly more serve during the crop season 2014 in comparison to 2013. The higher disease severity during the former year could probably be attributed to high rain fall, more number of rainy days and high relative humidity in 2014 as compared to 2013, especially during May to August which is conducive for the disease development

(Weekly metrological data of SKUAST-K, Shalimar for the year 2013 and 2014). Arauz and Sutton (1989a) [4], Beig (2006) [5] and Stephen Vann (2012) [28] who observed that warm wet conditions, high relative humidity and temperature of 30 °C were more conducive favouring disease development in apple and almond by *Botryosphaeria obtusa* and *Cryptosporiopsis* sp., respectively.

Extensive survey revealed that highest mean disease incidence (26.15%) and intensity (11.96%) of die-back was recorded in district Budgam and least mean disease incidence (19.24%) and intensity (8.78%) was recorded in district Pulwama. However, within locations highest disease incidence (34.57%) and intensity (15.89%) was recorded in Shelipora (district Budgam). It was followed by 29.33 and 13.45 per cent mean disease incidence and intensity, at Haproo of the same district. Least mean disease incidence (13.74%) and intensity (6.25%) was recorded at Kralchakh of district Pulwama. Similar trend was recorded in twig blight with highest disease incidence (19.85%) and intensity (9.12%) in district Budgam. However, lowest disease incidence (13.04%) and intensity (6.09%) in district Pulwama. Within locations highest disease incidence (26.27%) and intensity (12.28%) was recorded at Shelipora, and was followed by Haproo with incidence (23.07%) and intensity (10.52%). Least disease incidence (7.94%) and intensity (3.79%) was recorded at Kralchakh. These results are supported by the findings of Beig (2006) [5] and Bhat (2013) [6] who reported varying degree of incidence of *Cryptosporiopsis* sp. and *Diplodia seriata* in almond and pear, respectively. However, Khan (2010) [16] reported varying degree of incidence of both *Cryptosporiopsis* sp. and *Diplodia seriata* in apple.

Among the districts surveyed, the incidence and intensity of die-back disease based on mean of two years was higher in district Budgam (26.15% & 11.96%, respectively) followed by Pulwama (19.24 & 8.78%, respectively). Similarly the incidence and intensity of twig blight was also higher in district Budgam (19.85 & 9.12%, respectively) followed by district Pulwama (13.04 & 6.09%, respectively). The preponderance of old orchards with aged trees in district Budgam seem to have cumulative effect on disease development in these orchards. Lampinen *et al.* (2011) [19] also observed maximum vulnerability of aged almond trees to the attack of lower limb die-back disease. The faulty training and pruning practices observed in most of the orchards in the present study, in almond ecosystem at Budgam might have increased the frequency of occurrence of the die-back disease in these orchards. Rumbos (1997) [26], Slippers and Wingfield (2007) [27], Davison and Tay (2008) [11] also reported that faulty pruning, up-protected pruned ends and injuries culminated into high severity of stem canker and die-back diseases of *Grevillea robusta* initiated by *Botryosphaeria* spp.

Table 1: Incidence and intensity of die-back disease on almond (*Prunus amygdalus* Batsch.) in Budgam and Pulwama districts of Kashmir valley during 2013 and 2014

District	Location	Disease Incidence (%)*			Disease Intensity (%)*		
		2013	2014	Pooled	2013	2014	Pooled
Budgam	Haproo	27.33	31.33	29.33	12.64	14.26	13.45
	Nagam	21.87	24.80	23.34	10.00	11.21	10.61
	Nobug	16.13	18.67	17.40	7.38	8.53	7.96
	Shelipora	32.53	36.60	34.57	14.92	16.86	15.89
	Wahabpora	24.53	27.73	26.13	11.17	12.65	11.91
	Mean ± SD	24.48±14.17	27.82±16.06	26.15±15.12	11.22±6.67	12.70±6.99	11.96±6.83
	Range	16.13-32.53	18.67-36.60	17.40-34.57	7.38-14.92	8.53-16.86	7.96-15.89
Pulwama	Kralchakh	12.67	14.80	13.74	5.73	6.76	6.25
	Gasoo	19.87	22.40	21.14	9.00	10.25	9.63
	Kuil	15.60	17.73	16.67	7.19	8.12	7.66
	Rhoama	24.13	27.20	25.67	10.95	12.34	11.65
	Tahab	17.73	20.27	19.00	8.15	9.27	8.71
	Mean ± SD	18.00±10.16	20.48±11.90	19.24±11.03	8.20±4.71	9.35±5.24	8.78±4.98
	Range	12.67-24.13	14.80-27.20	13.74-25.67	5.73-10.95	6.76-12.34	6.25-11.65
Overall mean ± SD		21.24±12.17	24.15±13.98	22.70±13.08	9.71±5.69	11.03±6.12	10.37±5.91

*Average of three sites

Table 2: Incidence and intensity of twig blight disease on almond (*Prunus amygdalus* Batsch.) in Budgam and Pulwama districts of Kashmir valley during 2013 and 2014

District	Location	Disease Incidence (%)*			Disease Intensity (%)*		
		2013	2014	Pooled	2013	2014	Pooled
Budgam	Haproo	21.20	24.93	23.07	9.66	11.38	10.52
	Nagam	15.73	18.00	16.87	7.17	8.21	7.69
	Nobug	10.80	12.67	11.74	5.00	5.77	5.39
	Shelipora	24.40	28.13	26.27	11.38	13.17	12.28
	Wahabpora	20.13	22.53	21.33	9.17	10.28	9.73
	Mean ± SD	18.45±9.13	21.25±10.30	19.85±9.72	8.48±4.06	9.76±4.77	9.12±4.42
	Range	10.80-24.40	12.67-28.13	11.74-26.27	5.00-11.38	5.77-13.17	5.39-12.28
Pulwama	Kralchakh	7.60	8.27	7.94	3.64	3.94	3.79
	Gasoo	13.87	15.87	14.87	6.33	7.38	6.86
	Kuil	8.40	9.33	8.87	4.22	4.70	4.46
	Rhoama	18.27	21.87	20.07	8.38	10.00	9.19
	Tahab	12.67	14.27	13.47	5.77	6.53	6.15
	Mean ± SD	12.16±6.57	13.92±8.12	13.04±7.35	5.67±2.88	6.51±3.33	6.09±3.11
	Range	7.60-18.27	8.27-21.87	7.94-20.07	3.64-8.38	3.94-10.00	3.79-9.19
Overall mean ± SD		15.31±7.85	17.59±9.21	16.45±8.53	7.08±3.47	8.14±4.05	7.61±3.77

*Average of three sites

Of the villages surveyed, the highest incidence of die-back (34.57%) and intensity (15.89%) was recorded at village Shelipora of district Budgam, followed by Haproo, Wahabpora and Rhoama exhibiting disease incidence of 29.33, 26.13 and 25.67 per cent and intensity of 13.45, 11.91 and 11.65 per cent, respectively. Similarly the incidence and intensity of twig blight disease recorded in these villages was also highest. Higher disease occurrence in these villages could probably be attributed to higher plant density leading to higher relative humidity in the micro-climate of mono-cultured orchards, poor management practices besides, occasional rather neglected spray programme followed in these areas seem to have favored in building higher inoculums densities. The least disease incidence and intensity 13.74 & 6.25 and 7.94 & 3.79 of both the diseases was recorded in the village Kralchakh of Pulwama which could be attributed to better orchard management practices. Variation in incidence and intensity of almond twig blight disease in various locations have also been reported by Beig (2006) [5]. Varying degree of occurrence of twig and branch diseases of apple caused by *D. seriata* and *Cryptosporiopsis* sp. among various locations of Kashmir have also been observed by Khan (2010) [16].

Observations recorded on disease development with respect to symptomatology of die-back disease (Table 3) revealed that the appearance of initial disease symptoms in the first week of June, as light brown lesions on terminal bud scales of current season twigs as a result of which the infected leaf lets become water soaked, drooped and chocolate brown in colour. The observations are in conformity with the findings of Verma and Cheema (1984) [29] who also reported involvement of current seasons twigs in pear die-back caused by *Botryosphaeria* sp. Disease lesions, light brown in colour, then appeared on first 2-3 lateral buds in the 4th week of June which increased gradually in size.Elongation of these lesions lead to chlorosis and necrosis of terminal twig portion. Subsequently progression of chlorosis and necrosis in down-ward direction continued throughout the growing season resulting in shriveling and collapsing of the effected twigs. Gramaje *et al.* (2012) [14], Olmo *et al.* (2016) [20] also observed leaf chlorosis and twig necrosis of almond twigs due to die-back disease. The leaves over the diseased twigs turned chlorotic and defoliated prematurely, with the bark colour changing to dark brown. Small black pimple like elevations identified as the pycnidia of the fungus appeared on shriveled twigs towards the end of September which is in conformity with the findings of Verma and Cheema (1984) [29] who also observed pycnidial formation towards the end of season in die-back pear twigs. Further disease progression came to halt leavening a clear cut yellow margin between healthy and diseased portion.

Table 3: Symptomatological development of almond (*Prunus amygdalus* Bastsch.) twig blight disease

Period of observation (Month/Week)	Characteristic symptoms	Colour of diseased twig portion
Jun. 1st week	No symptoms	-
2nd week	Appearance of minute water soaked lesions on current seasons twigs mostly at the base of leaf petioles on mid to terminal twig portion	Light brown to brown
3rd week	Elongation of lesions along twig axis	-do-
4th week	-do-	-do-
Jul. 1st week	Elongated lesions appeared sunken	-do-
2nd week	Centre of the twig lesion becomes distinct Minute circular spots start appearing on leaves	-do-
3rd week	Lesions form elongated sunken streaks with raised margins	-do-
4th week	Wilting of leaves over the streak	-do-
Aug. 1st week	In some twigs centre of the lesions postulated	-do-
2nd week	Pin-head like structures (acervuli) formed on the centre of lesion	Brown to dark-brown
3rd week	Lesions encircle the twig at the point of iniciation	-do-
4th week	Complete girdling of twigs, however in some cases partial girdling was also observed Leaves above the girdled portion wilted/remain clinged	-do-
Sep. 1st week	Girdled portion appeared sunken and well demarcated from the healthy portion Leaf spots become distinct	-do-
2nd week	Necrosis progressed down the twig from girdled portion	-do-
3rd week	Necrotic extension came to halt in all the diseased twigs Sunken areas assume the shape of cankers with acervuli bark	-do-
Sept. 4th week onwards	No further advancement of the disease	-do-
Next season	No further progression of infection down the twigs	

*Figures based on mean of 25 observations

The twig blight symptoms (Table 4) appeared in the 2nd week of June, as small water soaked lesion at the base of leaf petioles mostly on mid to terminal twig portion. The lesions elongated along the twig axis both in up-ward as well as down-ward direction and assumed the shape of a streak, with sunken and raised margins. The streak usually encircled the twig at the point of inciation, which ultimately resulted in complete girdling of diseased twigs. However, in some twigs partial girdling was also observed. The leaves on girdled twigs appeared golden brown with distinct spots and remain clinged throughout the growing season unless disturbed by strong winds mechanically. The girdled portion of diseased twigs appeared well demarcated and sunken as compared to healthy portion. Towards the end of growing season i.e. in (3rd week of September) sunken areas on girdled twigs assumed the shape of cankers and further disease progression came to halt. Numerous pin head like structures identified as acervuli appeared sub-epidermaliy on the centre of cankers. The characteristic symptoms of the disease observed under natural conditions of inoculation were identical with those observed by Puttoo and Razdan (1988) [22], Chib (1994) [7] and Beig (2006) [5].

Table 4: Symptomatological development of almond (*Prunus amygdalus* Bastsch.) twig blight disease

Period of observation (Month/Week)	Characteristic symptoms	Colour of diseased twig portion
Jun. 1st week	No symptoms	-
2nd week	Appearance of minute water soaked lesions on current seasons twigs mostly at the base of leaf petioles on mid to terminal twig portion	Light brown to brown
3rd week	Elongation of lesions along twig axis	-do-
4th week	-do-	-do-
Jul. 1st week	Elongated lesions appeared sunken	-do-
2nd week	Centre of the twig lesion becomes distinct Minute circular spots start appearing on leaves	-do-
3rd week	Lesions form elongated sunken streaks with raised margins	-do-
4th week	Wilting of leaves over the streak	-do-
Aug. 1st week	In some twigs centre of the lesions postulated	-do-
2nd week	Pin-head like structures (acervuli) formed on the centre of lesion	Brown to dark-brown
3rd week	Lesions encircle the twig at the point of iniciation	-do-
4th week	Complete girdling of twigs, however in some cases partial girdling was also observed Leaves above the girdled portion wilted/remain clinged	-do-
Sep. 1st week	Girdled portion appeared sunken and well demarcated from the healthy portion Leaf spots become distinct	-do-
2nd week	Necrosis progressed down the twig from girdled portion	-do-
3rd week	Necrotic extension came to halt in all the diseased twigs Sunken areas assume the shape of cankers with acervuli bark	-do-
Sept. 4th week onwards	No further advancement of the disease	-do-
Next season	No further progression of infection down the twigs	

*Figures based on mean of 25 observations

Conclusion

It can be concluded that die-back an economically important disease of almond is prevalent in the almond growing areas of the valley and is a potential threat to almond plantation. Die-back and twig blight diseases appear on current season twigs during early June and progresses the as chlorosis and necrosis through the growing season.

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