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RESEARCH ARTICLE

ASSESSMENT OF DIFFERENT BRASSICA GERMPLASMS AGAINST *ALTERNARIA BLIGHT* AND ITS MANAGEMENT USING NATURALLY OCCURRING CHEMICALS

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Abstract

In the present study, sixteen advance lines/cultivars of *Brssica napus* and *Brassica juncea* were screened out against *Alternaria blight* of brassica (*Alternaria brassicae*). Out of eight lines/cultivars of *B. napus*, no line was found to immune against *A. brassicae*. Legend, Oscar, and Dgl were moderately resistant under field conditions and Shiralee was highly susceptible to *A. blight*. Similarly in *B. juncea*, no line was observed immune; Khanpur rya, Rya anmol, AUB 99 and SM 8300 were moderately resistant and B527-1 was highly susceptible to *A. blight*. In this study, three different plants extract (Ginger, Garlic and Turmeric) with 0.1%, 0.2% and 0.3% concentrations were evaluated against *A. blight*. The disease severity for all the plant extracts varied significantly at three different concentrations. Among these plant extracts, Turmeric (*Curcuma longa*) with 0.3% concentration gave significant result regarding disease intensity (12.74%) over control (26.86%). Similarly, these plant extracts with different concentrations were used to check their efficacy on yield (kg/ha) parameter. Turmeric with 0.3% concentration gave the satisfactory result to increase the yield (1545.33 kg/ha) as compare to untreated control (1086 kg/ha).

Introduction:-

Rapeseed (*Brassica napus* L.) is known as oilseed rape, rappi, rapa, and canola for specific group of cultivars. *Brassica juncea* L.is known as indian mustard, chinese mustard, mustard greens and sometimes leaf mustard. Both these species of Brassica are the members of the family brassicaceae and grown as oilseed crop in Pakistan [1]. Commercial canola (*B. napus*) oil has low erucic acid content and contains up to 0.56%-4.2% unlabeled trans fats. Trans isomers of α-linolenic acid, which are designed by deodorization of refined vegetable oils, can be found in substantial quantities in edible oils [12].

Total area under production of these two species in 2010-11 was 672 thousand acres in Pakistan. Area under production of Sarson, Poorbi raya, Taramira, Toria, and Raya is almost equally distributed in all parts of the country; only 30% of these crops are grown under barani and 70% is grown under irrigated conditions. During 2009-10 the total consumption was 2.9 million tons and the local production of edible oil was 662 thousand tons which was 23% of the domestic utilization and remaining 77% was imported to meet the requirement of the country [2].In spite of all the advancements in production technology, a large amount of the production is lost due to diseases and attack of insects. Diseases are one of the main factors affecting the *B. napus* and *B. juncea* production. Many pathogens attack the brassica and cause devastating diseases to plants at different stages of its growth. Many fungal and bacterial

diseases associated with this crop but *A. blight* of brassica (*A. brassicae*) is the important and ruthless disease of brassica crop around the world [9]. Attack of *A. blight* usually occurs on older leaves, stems, seedlings, and on adult plants at maturity stage. Dark circular spots increase with time that results in reduces photosynthetic rate of the leaves which causes immature ripening and poor quality seed is produced by the infected plants. Furthermore, pathogens may be transferred to other healthy plants as a result of infection from budding seeds which results in huge damage of stem and smaller younger plants under high relative humidity where large number of close seedling grows [7].

A. brassicae and *A. brassicola* have the potential to attack the host species at any stage of development; it can also attack on seeds. Dark stem lesion brown or black in color appears on seedling after germination that may lead to damping off and less growth of plants. Valkonen et al., [15] suggested that the older plants are more susceptible to *A. blight* because they are more close to soil and they are easily attacked by the pathogen as a result of transfer of pathogen through wind or rain splash [3].

Primarily, scouting, sanitation, developments of resistant varieties and fungicides are used to control *A. blight*. Fungicides are widely used by the farmers. Agrochemicals like fungicides lead to serious environmental threats [6, 10]. Keeping in view these facts about this disease, the objective of this research was to focus on isolation, purification and identification of the fungal pathogen associated with diseased samples; mass culturing of *A. brassicae*; screening of brassica germplasm against leaf spot disease and in vivo evaluation of different plant extracts against the *A. blight*.

Materials and Methods:-

Leaves sample of *B. napus* and *B. juncea* were taken from diseased as well as healthy plants. These samples were collected from oilseed research area of Ayub Agriculture Research Institute (AARI), Faisalabad. Potato Dextrose Agar (PDA) was used for isolation and multiplication of fungal pathogen. One liter medium of PDA was prepared according to Javed et al., [5] method.

The infected samples consisting of leaves were cut into small pieces of 4-5 mm length and these pieces were surface sterilized with 1% sodium hypo chloride for 1-2 min. These pieces were rinsed with distilled water twice before transferring to sterilized filter paper in petri plates for drying. Sterilized pieces were placed on petri plates containing PDA media with the help of sterilized forceps. Petri plates were incubated at 27 ^{0}C for 5-7 days and were observed on daily basis. The isolated fungus were examined under microscope .The identification of fungi was done by observing morphological characters and description; then fungus colonized on these pieces were purified. For screening of *B. napus* and *B. juncea* against *A. blight*, seeds of different cultivars/advance lines were collected from oil seed research institute of AARI, Faisalabad and Department of Plant Breeding and Genetics, University of Agriculture Faisalabad. These lines of brassica were sown in research area of Department of Plant Pathology, University of Agriculture Faisalabad on 22-November 2010 under RCBD design with three replications.

The inoculum of *A. blight* (on nutrient broth in distilled water) was prepared and converted into suspension with the help of shaker. This spore suspension (inoculum) was applied on *B. napus* and *B. juncea* plants by spray method with the atomizer after spores counting with heamocytometer.

Severity of disease was recorded 10 days after the inoculation of crop. Data regarding the disease severity was taken three times; (1) after appearing symptoms, (2) after 75 days and (3) after 90 days respectively. At each time interval, five plants of each line/cultivar were selected for assessment of disease severity of *A. blight*. Disease severity percentage was recorded by using the formula suggested by Sangeetah et al., [11](Table 1).

$$\text{Disease severity} = \frac{Infected\ leaf\ area}{Total\ leaves\ of\ plant} x\ 100$$

Table 1: Scale of disease severity according to symptoms.

Scale	Description of symptom
0	Leaves free from infection
1	Spots covering <5% leaf area
2	Spots covering 5.1-10% leaf area
3	Rings covering 10.1-25% leaf area
4	Blight symptoms covering 25.1-50% leaf area
5	Blight symptoms covering >50% leaf area

The management of disease was done by using different plant extracts viz ginger, garlic and turmeric. These plant extracts were applied to the crop with three different concentrations 0.1%, 0.2% and 0.3%. These plants extracts were used for controlling the disease intensity and their efficacy on yield (kg/ha) was also recorded.

Results and Discussion:-

Field screening of *B. napus* and *B. juncea* germplasm against *A. blight* showed highly significant results. Data regarding the percentage of disease severity during different growth stages have been presented in the Table 2.

Dunkled, Bulbul, Cyclone, and Rainbow of *B. napus*, were found to be moderately susceptible against the *A. blight*. Legend and Oscar were moderately resistant while Shiralee was highly susceptible amongst all the cultivars/lines of *B. napus*. Statistical analysis showed that significant increase in disease severity was observed after 90 days of sowing in most of the cultivars/lines of *B. napus*. Disease severity increases with the passage of time. Shiralee showed the significant increase in disease severity from 60 (67.15%) to 90 (80.5%) days interval respectively. Shiralee showed the highest disease severity amongst all the cultivars of *B. napus.* Oscar showed the minimum disease severity (8.98%) amongst all the cultivars/lines. Comparative analysis of all the eight cultivars/lines of *B. napus* showed that, no line/cultivar was immune and resistant against the *A. blight* of Brassica. Legend, Oscar, and Dgl were moderately resistant against *A. blight* and these cultivars showed the minimum disease severity. Four cultivars/lines (Dunkled (21.34%), Bulbul (17.33%), Cyclone (25.10%) and Rainbow (23.43%) were moderately susceptible against *A. blight* (Table 2&3).

Table 2: Percentage ± SE of disease severity of *Brassica napus* after different time interval

Cultivars/lines	60 Days	75 Days	90 Days	Overall Means
Dunkled	13.9±0.004	22.10±0.03	27.96±0.332	21.34±0.023
Bulbul	11.72±0.141	18.22±0.132	22.06±1.112	17.33±1.12
Cyclone	12.37±0.242	26.23±1.242	36.1±0.342	25.10±1.234
Dgl	4.98±0.032	9.61Tv0.312	12.98±0.942	9.19±0.055
Rainbow	16.63±1.264	22.69±1.112	30.30±1.643	23.43±1.24
Shiralee	67.1±2.13	79.70±0.243	80.5±2.34	75.78±2.235
Oscar	5.09±0.032	10.55S±0.232	11.32±0.235	8.98±0.0432
Legend	5.06±0.143	9.70±0.342	14.06±0.342	9.60±0.034

Table 3: Response of *Brassica napus* line/cultivars against *A. blight*

Varieties/Lines	Disease severity (%)	Response
Dunkled	21.34[a]	Moderately Susceptible
Bulbul	17.33[e]	Moderately Susceptible
Cyclone	25.10[b]	Moderately Susceptible
Dgl	9.19[f]	Moderately Resistant
Rainbow	23.43[c]	Moderately Susceptible
Shiralee	75.780[a]	Highly Susceptible
Oscar	8.985[gh]	Moderately Resistant
Legend	9.60[fg]	Moderately Resistant

Means sharing the same letters are not significantly different according to LSD at α=5%

While in-case of *B. juncea*, disease severity was observed minimum in case of Khanpur rya, Rya anmol, and SM 8300. All these cultivars were moderately resistant against *A. blight*. According to scale, Comet and 20-E found to be susceptible against *A. blight*. Maximum percentage of disease severity was recorded in B527-1. Maximum disease severity percentage was observed after 90 days of sowing in most of the cultivars/lines of *B. juncea*. Comparative analysis of all the eight cultivars/lines of *B. juncea* showed that, no line/cultivar was immune and resistant against *A. blight*. Four lines were moderately resistant against *A. blight*. Khanpur rya (8.093%), Rya anmol (8.45%), AUB 99 (9.79%) and SM 8300 (8.615%) showed the minimum disease severity percentage. B527-1 was highly susceptible to *A. blight* with disease severity (75.69%) (Table 4&5).

Table 4: Percentage ±SE of disease severity of *Brassica juncea* after different time interval

Cultivar/lines	60 Days	75 Days	90 Days	Overall Means
Khanpur rya	4.57±0.043	8.54±0.472	11.06±0.472	8.09±0.837
Rya anmol	4.70±0.035	9.45±0.485	11.47±0.485	8.45±2.583
B527-1	65.39±2.144	75.30±2.275	86.39±2.46	75.69±2.35
AUB 99	5.20±0.045	10.30±0.884	12.98±1.253	13.87±0.384
P 13-2	14.65±1.354	18.84±0.785	22.57±1.364	18.24±0.849
Comet	31.06±0.758	41.95±1.674	52.06±2.125	41.69±1.894
20-E	36.43±1.124	45.79±1.246	11.32±0.946	48.57±2.135
SM 8300	5.28±0.984	9.09±0.894	11.56±0.742	8.15±0.424

Table 5: Response of *Brassica juncea* line/cultivars against *A. blight*

Sr. .No	Verieties/lines	Disease Severity (%)	Response
1	Khanpur rya	8.093[e]	Moderately Resistant
2	Rya anmol	8.457[e]	Moderately Resistant
3	B527-1	75.693[a]	Highly Susceptible
4	P13-2	18.24[d]	Moderately Susceptible
5	AUB 99	9.790[e]	Moderately Resistant
6	Comet	41.690[b]	Susceptible
7	20-E	43.030[b]	Susceptible
8	SM 8300	8.615[e]	Moderately Resistant

Means sharing the same letters are not significantly different according to LSD at α=5%

By the application of different plant extracts, the percentage of disease intensity varied at different concentrations viz 0.1%, 0.2% and 0.3%. Ginger and garlic extracts were least effective in controlling the fungal infections; which corresponded to 19.98% and 17.37% intensity respectively. Therefore, turmeric extract seems effective and could be used by farmers in controlling seed-borne fungi.

Turmeric extract proved to be the most effective for control of brassica at 0.3% concentration with minimum disease intensity (13.58%) as compare to other treatments. This showed that, efficacy of different plant extracts can improve by increasing the concentration level. The plant extracts had profound effect to reduce the disease severity of brassica species. In case of plant extracts (ginger, garlic and turmeric) the overall mean of percent disease intensity varied 19.98%, 17.37%, and 13.58% (Table 6) respectively.

Table 6: Efficacy of different plant extracts against *A. blight* under field conditions.

Treatments	Disease intensity (%) at different concentrations			
	0.1%	**0.2%**	**0.3%**	**Overall Means**
Ginger	20.4[b]	18.53[c]	21.03[b]	19.98[b]
Garlic	17.78[c]	17.2[c]	17.15[c]	17.37[c]
Turmeric	14.04[d]	13.97[e]	12.74[f]	13.58[d]
Control	26.86[a]	26.86[a]	26.86[a]	26.86[a]

Means sharing the same letters are not significantly different according to LSD at α=5%

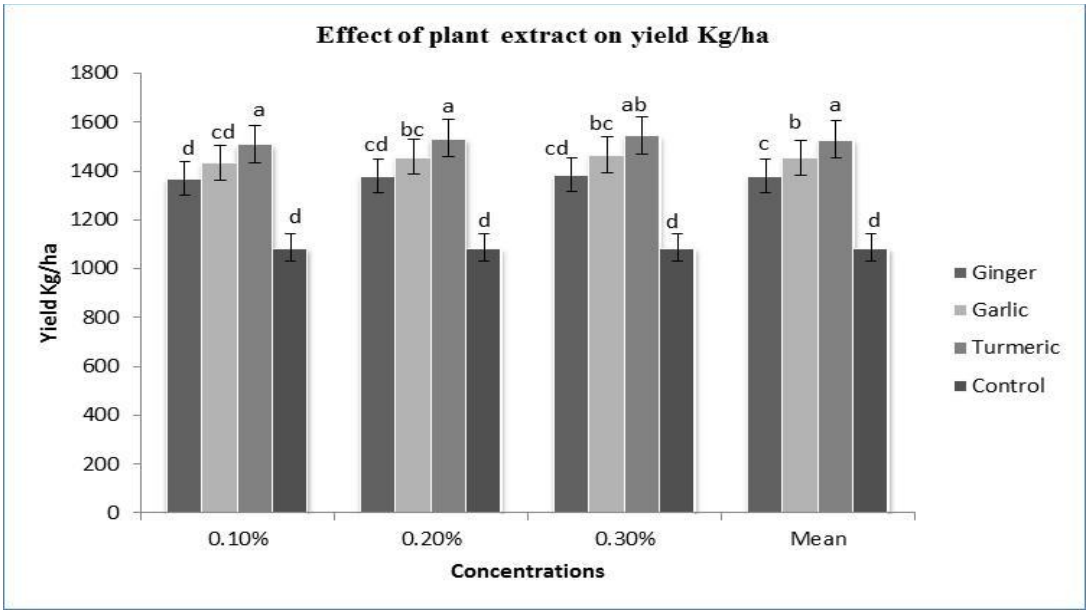

Figure 1: Effect of different concentrations of plants extracts on yield kg/ha.

Pod yield can be increased by proper and timely application of plant extracts. Maximum yield (kg/ha) was obtained by the application of plant extracts as compared to control treatment. In contrast of plant extracts, turmeric gave maximum yield of brassica crops as compared to other plant extract (Fig. 1). In order to harp the remunerations of induced host resistance and figure out a stable, long term resistance mechanism into host plant against the pathogen, there is a need to identify the pathogen and understanding its behavior under different conditions. Our results showed that, Legend and Oscar were moderately resistant while Shiralee was highly susceptible amongst all the cultivars/lines of *B. napus*. While in-case of *B. juncea*, disease severity was observed minimum in case of Khanpur rya, Rya anmol, and SM 8300. B527-1 was highly susceptible to *A. blight*. The oilseed crops, *B. napus*, *B. carinata* and *S. alba* have relatively more epicuticular wax than *B. rapa* and *B. juncea* and tend to be less sensitive to *A. blight*. Resistance of rapeseed-mustard against *A. blight* is also found to be related with many factors like deterrence to conidial retention on surface of plant like high deposits of epicuticular wax that result a physical obstacle as a hydrophobic coating to decrease deposition of water-borne inoculum [4,14].

Fungicide remained most effective to control diseases in plants, but increasing public concern about environmental hazard, fungicide are no more considered as a suitable technique to manage plant diseases. The results of present study showed that, turmeric extract proved to be the most effective against *A. blight* of brassica at 0.3% concentration. Many scientists worked on control Alternaria leaf blight of brassica by different plant extracts and chemicals. Our results are in agreement with [8, 13] who checked the efficacy of plant extracts obtained from garlic, neem leaf, ginger and onion bulb against *Alternaria brasicae*. It was observed that the application of plant extracts show significant result to increase the seed germination as compare to untreated control.

Conclusion:-

Out of eight lines/cultivars of *B. napus*, Legend, Oscar, and Dgl were moderately resistant and Shiralee was highly susceptible to *A. blight*. Similarly in *B. juncea*; Khanpur Rya, Rya anmol, AUB 99 and SM 8300 were moderately resistant and B527-1 was highly susceptible to *A. blight*. Sowing of crop at proper time and temperature will also help to escape diseases. There is a need to use varieties/ lines that must be resistant to this disease in order to avoid any epidemic in future. For the management of *A. blight* and to obtain maximum yield, turmeric can be used in brassica crop.

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