

Occurrence and life cycle of broomrape (*Phelipanche aegyptiaca* Pers.) on mustard (*Brassica campestris* L.) in Northeastern regions of Rajasthan

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ABSTRACT

Broomrape (*Phelipanche* and *Orobanche*) weeds are parasitic on a variety of dicotyledonous plant species, severely harming important cash crops all over the world. The process of broomrape infestation in mustard fields (oilseed crop) is poorly understood in Rajasthan. Over the past few years, the number of broomrape-contaminated mustard fields has increased in many regions of Rajasthan. Field surveys were conducted to investigate the occurrence of broomrape in mustard-growing areas of northeastern Rajasthan. Field studies were also carried out in the extensively broomrape-infested fields in the Nawalgarh region of Jhunjhunu (Rajasthan, India) (27°51'0.00" N 75°16'12.00" E) to evaluate the life stages and severity of broomrape infestation. *Phelipanche aegyptiaca* Pers. (Egyptian broomrape) was found infesting the fields of *Brassica campestris* L. Field experiments were conducted to record all the underground and aerial life stages of *P. aegyptiaca*. Among all the visited plots, the Mukandgarh region exhibited the highest incidence and severity of *P. aegyptiaca* during the emerging, flowering, and fructification stages, whereas the Nawalri region displayed the lowest incidence and severity at each stage. Mustard yield and quality were severely affected by the *Phelipanche* infestation. With an average of 50% disease incidence in most of the plots, flowering and fructification stages of broomrape are the most difficult to control and cause 100% infestation to the crop. The interrelationship of *P. aegyptiaca* and *B. campestris* is not studied extensively, especially in Rajasthan. To date successful control of this weed is not achieved. The present investigation will help understand the histological interactions of *P. aegyptiaca* on *B. campestris* about days of sowing (DAS). These interactions will certainly help to design cultural and biological control strategies by determining the most effective timing of control concerning the timeline of germination and infestation of *P. aegyptiaca*.

1. INTRODUCTION

Plant parasitic floras are considered one of the most damaging biotic constraints that pose a serious threat to agricultural production. Parasitic plants consisted of 1% of all angiospermic plant species [1]. There are 20 dicot families, including 4,000 plant species that parasitize other angiosperms [2]. Of the various parasitic dicot families, Orobanchaceae is the most widespread and damaging parasitic angiospermic plant family. Broomrape (*Orobanche* and *Phelipanche*) is the largest genus among all genera of Orobanchaceae. The broomrape genus consists of more than 100 species, some of which are very noxious to many economically important crops [3]. Broomrape is a holo root parasite

and angiospermous plant, devoid of chlorophyll and entirely depends on the host plant for supply of water, carbon, nitrogen, and inorganic solutes [4].

Broomrape species occur worldwide including in major regions such as Asia, Europe, and North Africa [2]. In India, the wide distribution of broomrape occurs in the states of Andhra Pradesh, Madhya Pradesh, Rajasthan, Gujarat, western Uttar Pradesh (UP), Punjab, Haryana, Karnataka, Tamil Nadu, and Maharashtra [5]. Broomrape has been observed in India as a rapidly increasing threat to rapeseed–mustard production [6]. States that produce a majority of mustard such as northern Rajasthan, western UP, Haryana, northeast Madhya Pradesh, and Punjab are heavily infested with broomrape [7]. In parts of Rajasthan (Jhunjhunu) and Haryana states, broomrape causes huge losses due to serious infestation of mustard crop [8]. Broomrape affects a variety of crop plant species, including mustard, sunflower, brinjal, potato, tomato, tobacco, cabbage, turnip, and cauliflower [9].

Mustard is an important group of edible oilseeds cultivated in India and found severely infested with broomrape. India ranks fourth in

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terms of production (10.37%) of oilseed (*Brassica* sp.) after the European Union, Canada, and China in the world. Mustard ranks second after soybean in the oilseed crop production in India. It accounts for 23.91% area and 27.19% production of the total oilseeds in the country. Rajasthan holds the first position in India for mustard cultivation by contributing 41.44% acreage and 45.03% production [10]. In Rajasthan, Jhunjhunu stands out as a promising district, boasting a growing area of 75,054 hectares, yielding a production of 116,979 tons, and achieving a productivity of 1,559 kg per hectare for rapeseed–mustard [11]. Broomrape causes great damage and becomes an economic and ecological problem in mustard-cultivated areas of Rajasthan. It is difficult to manage because of its late emergence, large number of microscopic seeds, and subterranean location in mustard fields. Yield reduction in mustard can range from 10 to 58% depending upon the intensity of weed infestation [12].

Broomrape is well known for economic losses in crop production. Labrousse and Delmail [13] named “the vampire world” to the parasitic plants due to their detrimental effects. Parasitic weeds can cause a reduction in growth and yield to complete deterioration of the host crop. Since these parasites cause maximum damage before the broomrape’s shoots develop and blossom, controlling them with pesticides or other methods is unsuccessful. As a result, the majority of crop losses could happen before an infection is identified. Many studies suggest that broomrape causes a heavy economic loss in many regions of the world. Broomrape can cause up to 100% production losses depending on the crop plant, environmental factors, and infestation level [4]. The entire losses brought on by all other agricultural production constraints and the average yield reduction from broomrapes alone represent about 30% [14].

Several studies on broomrape control or its infestation in relation to various mustard species have been performed in Rajasthan. Lal et al. [15] investigated the chemical control over *P. aegyptiaca* infestation in *Brassica juncea* fields of the Ajmer district. Akhter and Khan [16] tested the resistance of different genotypes of *B. juncea* against *P. aegyptiaca*. Singh et al. [17] applied an integrated approach to control broomrape via chemical, botanical, and cultural methods in Jhunjhunu and Bikaner districts of Rajasthan, India. Shukla [18] performed a screening test of 250 *B. campestris* var. *toria* genotypes and few were found effective against *P. aegyptiaca*. The life cycle of broomrape with *B. campestris* has not been studied earlier in Rajasthan. The present study was conducted to identify broomrape species that infested *B. campestris*, to study the broomrape life stages, and to reveal broomrape incidence and severity in the *B. campestris* fields of Jhunjhunu (Rajasthan). In recent years, several researchers have emphasized the necessity of expanding our understanding of parasitic plant biology to make it simpler to create appropriate procedures for effective management [19]. The present study will present a suitable timeline to design management strategies for this particular crop species. The most effective management can be suggested to farmers only after studying the below-ground and above-ground stages of broomrape.

2. MATERIALS AND METHODS

The largest state in India, Rajasthan, is located between latitudes 23°3' and 30°12' North and longitudes 69°3' and 78°17' East, respectively. Jhunjhunu district is located in the northern plain of the state of Rajasthan. The field studies were performed multiple

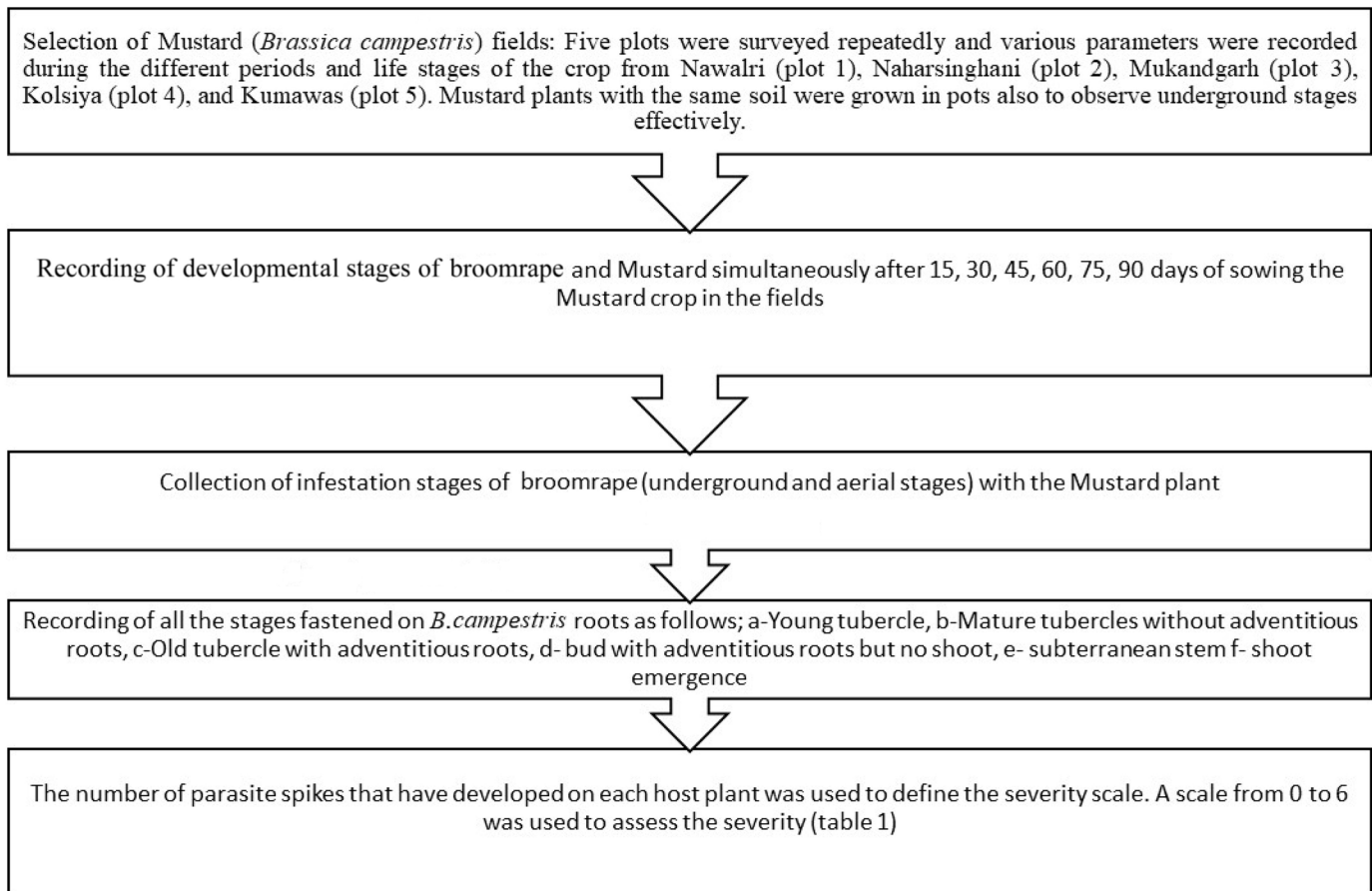
times in many field plots in the Nawalgarh region of Jhunjhunu district, Rajasthan (27°51' N 75°16' E). This region used to have an average (30 years) yearly rainfall and relative humidity of 457 mm and 45.62%, respectively, with a 25.2°C average temperature. To investigate different stages of the broomrape life cycle, evaluations of both naturally occurring infestation in the field and controlled infection in pots were carried out. Mustard plants were also grown in pots with the same soil to observe underground stages effectively. Pot experiments were performed parallel to the field studies. The experiments were carried out till the first broomrape shoot emerged. *Brassica* plants along with parasitic stages were taken at six different states, i.e., 15, 30, 45, 60, 75, and 90 DAS (days after sowing) of *Brassica* plants. At each sampling, the number of infections per plant and different parasitic stages were observed. Broomrape infestation level was determined by observing the number of *P. aegyptiaca* phases fastened on *B. campestris* roots as follows: (a) young tubercle, (b) mature tubercles without adventitious roots, (c) old tubercle with adventitious roots, (d) bud with adventitious roots but no shoot, (e) subterranean stem, and (f) shoot emergence [19].

Field surveys have been conducted on broomrape infesting mustard fields located in different villages of Nawalgarh during the winter season of 2021–22. Five plots were surveyed repeatedly and various parameters were recorded during the different periods and life stages of the crop from villages, including Nawalri (plot 1), Naharsinghani (plot 2), Mukandgarh (plot 3), Kolsiya (plot 4), and Kumawas (plot 5). In field studies, the emergence (number per plant) of broomrape shoots concerning *B. campestris* plants and the average shoot height of both were recorded. The impact of broomrape parasitism as a reduction in fresh and dry matter of *B. campestris* plants was also recorded in each field. These surveys were performed many times (emerging, flowering, and fructification stages) during the growing season of the mustard crop to investigate different aerial broomrape life stages. During the survey, heavily infested *B. campestris* plants, broomrape plants, broomrape seeds, and various stages of host–parasite colonization were collected. During the survey, the parasite incidence I (proportion of plants with emerging broomrape stems) and severity S (on a scale of 1–6) were also determined. The number of parasite spikes that have developed on each host plant was used to define the severity scale. A scale from 0 to 6 was used to assess the severity [Table 1] [20].

Table 1: Broomrape distribution evaluation using the severity scale in the Jhunjhunu area.

| Rating Scale | Definition | Infestation |
|--------------|---|---|
| 0 | No broomrape shoot occurred | No infestation |
| 1 | Very few number of broomrape shoots in the entire field | Very low |
| 2 | Few number of broomrape shoots in the entire field | Low |
| 3 | Most of the host plants have two broomrape shoots | Moderate |
| 4 | More than two broomrape shoots on the host plant | High |
| 5 | More than five broomrape shoots on every host plant | Very high |
| 6 | Dead host plants, produce nothing | Complete destruction of the host plants |

Scheme of Experimentation



3. RESULTS

P. aegyptiaca Pers. was the species of broomrape identified in the *B. campestris* fields of Rajasthan that were assessed (RUBL 211776). Locally, broomrape is termed as margoja, gulli, khumbhi, or rukhri. Branching, yellow-brown, glandularly hairy stalks with blue blooms that range in color from blue to violet define broomrape.

3.1. Underground Developmental Stages of *P. aegyptiaca* Pers

When the first sample was taken at 15 DAS, *Brassica* roots were not infected. In the experiment, the continuous observations suggested that after 30 days of sowing the tubercle stages started developing on the host roots [Figures 1A and 1C], which increased in number with time [Table 2]. The *Phelipanche* tubercle develops at the penetration point of the host surface and becomes a storage organ. The strong sink activity of the tubercle converts it into a bulbous structure.

Buds were observed after 45 DAS [Figures 1D–G]. *P. aegyptiaca* development did not go further than the young tubercles, old tubercles, and bud stages throughout the 70 DAS-long experiment [Table 2]. It takes very little time to develop an old tubercle into a bud. These buds further give rise to the shoots [Figures 1H–J]. These shoot outgrowths (spikes) become visible above the ground after 3–4 weeks, which further gives rise to flowers. At the time of shoot emergence, most of the damage has already occurred. The first underground stem was observed at 75 DAS. The underground shoots were going through an elongation process and emerged above the ground after 90 DAS. The

P. aegyptiaca plant body was divided into shoot, root, and tuber parts. The short, branched stem rose as a protuberance above the ground. It possessed leaves that were devoid of chlorophyll.

3.2. Aerial Stages of *P. aegyptiaca* Pers

At the end of October month, plant material *B. campestris* seeds were sown in fields. The field experiment was carried out till the *Brassica* crop was harvested. In the field, *Brassica* plant emergence occurred 4–6 DAS. When the field observations were performed at 30, 40, and 50 DAS of *Brassica*, there was no shoot emergence above the ground. The first *P. aegyptiaca* stem emergence at the soil surface was taking place 60 DAS [Figure 2A] (i.e., emerging or early flowering stage). *P. aegyptiaca* stems were thin, simple, pubescent-glandular, erect, purplish white, and roundish in morphology [Figure 2B]. They grow near the host stem base [Figure 2C] or far from it, depending on the length of their host root system. A parasite shoot could be found associated with that plant up to 40 cm away from the crop plant [Figure 2D]. The soil was removed 2–3 cm from the ground, and a large number of underground stems remained ready to emerge [Figure 2E]. It was found that a single *Brassica* plant can be infected with up to 20 *Phelipanche* plants [Figure 2F].

Once the *Phelipanche* plant reached 4–5 cm in height, it started flowering and turned into a flower scape [Figure 3A]. *Phelipanche* shoots emerged either single or in clusters [Figures 3B and 3C]. This phenomenon occurred within 3–4 weeks after shoot emergence. The flowers were purple colored and 1–1.5 cm in length. Each *Phelipanche* plant might have up to 15–20

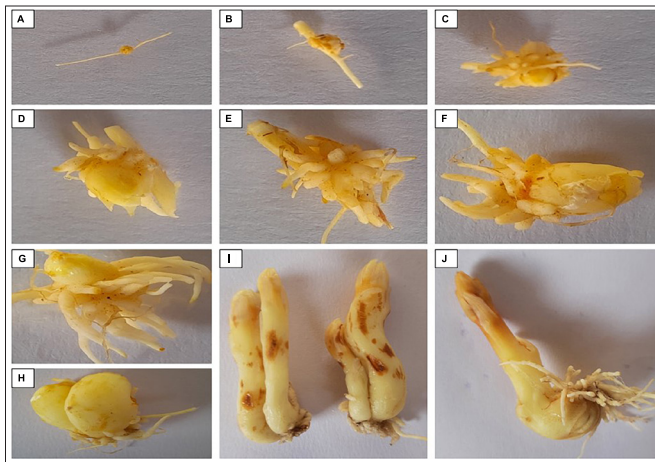


Figure 1: The underground life stages (from tubercle to shoot formation) of *Phelipanche aegyptiaca*: (A and B) young and mature tubercle at 30 DAS, (C) tubercle with adventitious roots at 45 DAS, (D–G) bud with adventitious roots but without stem between 45 DAS and 75 DAS, (H and I) underground stems at 90 DAS, and (J) first emerged stem after 90 DAS.

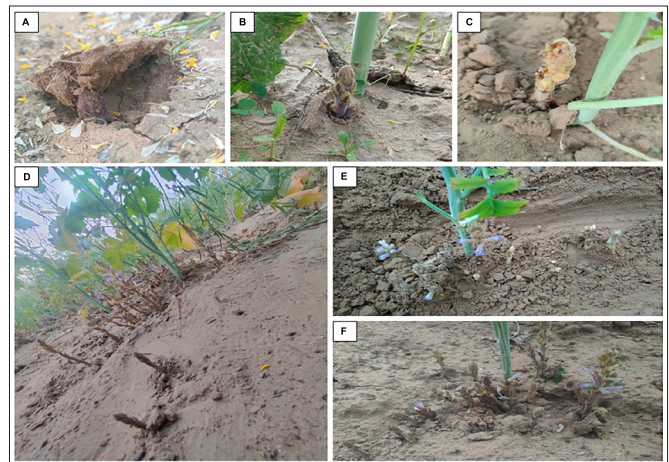


Figure 2: Above-ground shoot emergence stages: (A–C) *P. aegyptiaca* shoot emergence as a round, glandular protuberance above the ground, (D) connection between *B. campestris* and *P. aegyptiaca* up to 40 cm distance, (E) *B. campestris* with *P. aegyptiaca* under- and above-ground shoots, and (F) infestation of many *P. aegyptiaca* shoots on a single *B. campestris* plant.

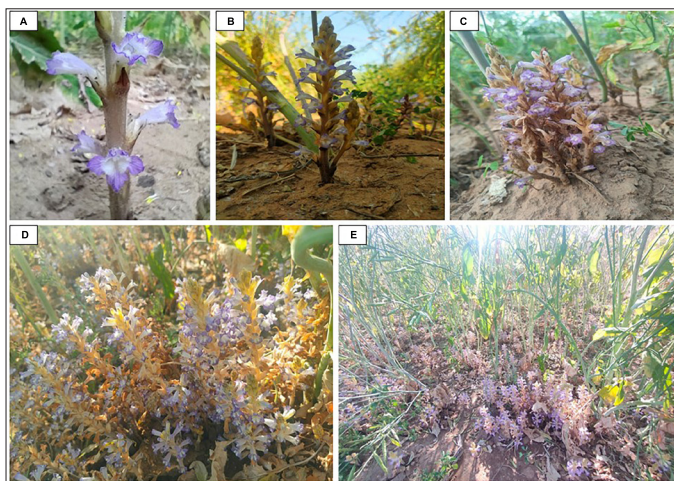


Figure 3: *Phelipanche aegyptiaca* flowering stages: (A and B) single broomrape plant with purple flowers, (C) *P. aegyptiaca* shoots in the cluster, and (D and E) heavily *P. aegyptiaca* infested mustard plots.

flower scapes in cases of severe infestations; at that point, the affected area looked to be completely covered with *P. aegyptiaca* [Figures 3D–E].

The *Phelipanche* shoots continuously emerged until the end of the growing season of *Brassica*. The shoots were found either branched (from the middle point) or unbranched. *P. aegyptiaca* shoots were crowded with scaly leaves and purple flowers. Leaves were broad at the base and pointed at the tip and alternatively arranged on the stem. Flowering started in January and ended in the first week of March. Flowers were represented by light yellow calyx, purple corolla, stamen, and ovary. Flowers were replaced by capsules [Figures 4A and 4B] after pollination as the calyx and corolla parts were withered. The *Phelipanche* plants were having 15–35 cm in height with an average of 25 cm. Usually, the areas on the edges of the fields were the worst affected.

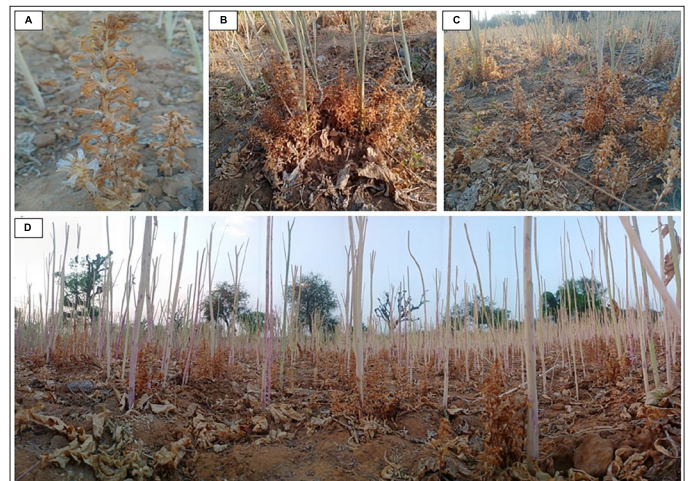


Figure 4: *Phelipanche aegyptiaca* fructification stages: (A and B) completely mature dried *P. aegyptiaca* plants with capsules, (C) *P. aegyptiaca* plants that start degrading after crop harvesting, and (D) clearly visible *P. aegyptiaca* plants aligned with the growing lines in crop harvested field.

Each *Phelipanche* plant consisted of up to 20 capsules and every capsule consists of thousands of whitish seeds, which turn black after maturation. During dehiscence, the dried capsules become split, and completely dried and mature black tiny seeds fall off and increase the seed bank every year in the soil. The huge seed bank of *P. aegyptiaca* remains in the soil for years. When the *Brassica* crop becomes mature and starts drying [Figures 4C and 4D], the parasite plant body also starts degrading. This degradation process starts from the root part. First, the adventitious roots started the necrosis at the tip of the end.

Thus, *Phelipanche* consisted of several below-ground and above-ground ontogenic development stages [Figure 5]. Major stages are seed germination, host penetration, and stalk emergence followed by flower formation and seed generation.

Table 2: Number of *P. aegyptiaca* attachments recorded on infested *B. campestris* root systems during every observation.

| Dates of Observation | Young Tubercle | Mature Tubercle Without Adventitious Roots | Tubercle With Adventitious Roots | Bud With Adventitious Roots But Without Stem | Underground Stem | Shoot Emergence | Total Number of Attachments |
|----------------------|----------------|--|----------------------------------|--|------------------|-----------------|-----------------------------|
| 15 DAS | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 DAS | 7 | 2 | 1 | 0 | 0 | 0 | 10 |
| 45 DAS | 19 | 11 | 7 | 0 | 0 | 0 | 27 |
| 60 DAS | 25 | 18 | 19 | 16 | 0 | 0 | 78 |
| 75 DAS | 21 | 14 | 32 | 19 | 4 | 0 | 90 |
| 90 DAS | 17 | 33 | 35 | 31 | 10 | 1 | 127 |

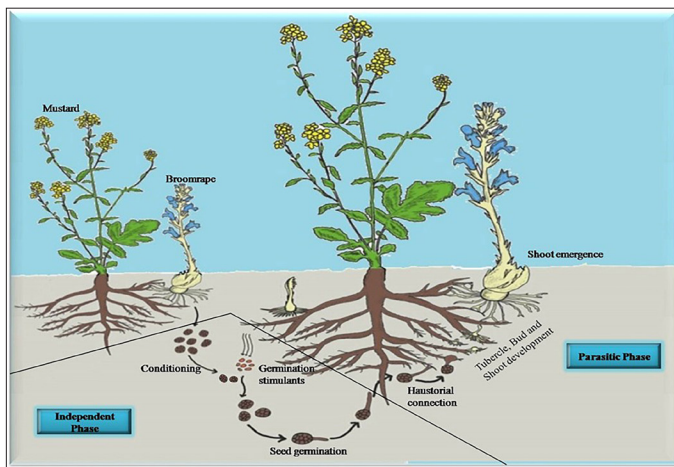


Figure 5: Life cycle of root parasitic plant *Phelipanche* comprises different above- and below-ground life stages. The underground stages include seed preconditioning and seed germination, which represent the independent phase whereas haustoria development, tubercle and bud formation, and shoot development stages reflect parasitic phase of broomrape life cycle.

3.3. Disease Incidence and Severity

Data were collected from five fields located in different regions of Jhunjhunu such as Nawalri (plot 1), Naharsinghani (plot 2), Mukandgarh (plot 3), Kolsiya (plot 4), and Kumawas (plot 5). In each plot, the number of emerging *Phelipanche* per plant served as a measure of *P. aegyptiaca* infection. The survey was conducted three times in the winter growing season of 2021–22. In the first week of January, the emerging stage of *Phelipanche* took place, while the *Brassica* crop completed its vegetative phase. During the survey in the first week of February, the *Phelipanche* plants showed flowering while in March when the crop was ready to harvest, *Phelipanche* plants showed the fructification stage and started drying. Incidence of *P. aegyptiaca* attack ranged from 5 to 100% [Figure 6A]. The incidence percentage reached 50% of the average in the emerging stage at all plots except plot 1. At plot 2 and plot 3, *Phelipanche* incidence extended to 100% at only the flowering stage while the other plots are less infected. During the fructification stage, every plot showed 100% *Phelipanche* incidence except plot 1. In plot 2 and plot 3, *P. aegyptiaca* was found to have the maximum severity, scoring 4 out of 6, whereas plot 1 showed minimum severity [Figure 6B]. In the maturation or fructification phase, the frequency of *P. aegyptiaca* was the greatest. *Phelipanche* prevalence differs throughout the investigated regions. Plot 3 showed the maximum reduction in fresh and dry matter of the

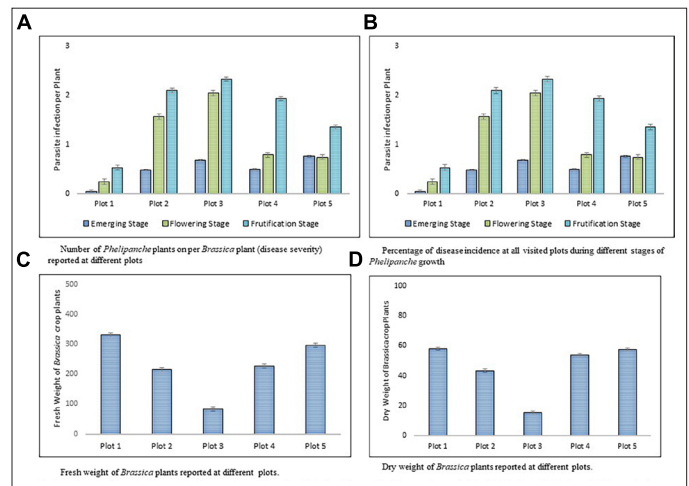


Figure 6: *Phelipanche* disease incidence, infection severity, and host plants' fresh and dry weight (shoot+root) recorded in all field plots. (A) Number of *Phelipanche* plants on per Brassica plant reported at different plots, (B) percentage of disease incidence at all visited plots during different stages of *Phelipanche* growth, (C) fresh weight of Brassica crop plants reported at different plots, and (D) dry weight of Brassica crop plants reported at different plots (Nawalri [plot 1], Naharsinghani [plot 2], Mukandgarh [plot 3], Kolsiya [plot 4], and Kumawas [plot 5]).

host and the highest infection severity, whereas plot 1 exhibited just a little infestation of *P. aegyptiaca* [Figures 6C and 6D].

4. DISCUSSION

The absence of any kind of infection at 15 DAS during pot observation [Table 2] means that the essential germination signals for the parasite may be secreted by the host roots between 15 and 30 DAS. Stimulant-induced germination results in a germ tube or radicle emergence that is likely to establish a connection to the plant. The radicle elongates toward the host roots by using the chemical gradient. The terminal end of the radicle redifferentiates into a haustorium. It happens when the apical cell develops in intrusive cells that penetrate the host surface [21]. Phenols, quinones, and plant hormone cytokinin have been identified as signal molecules to direct the radicle growth toward the host. The penetration may result from enzymatic activities such as polygalacturonase, endonuclease pectin, and methyl esterase [22]. After host penetration, the haustorium acts as a bridge between the parasite and the host vascular system. This connection allows the parasite to extract various substances. The mainly transferred assimilates from host to parasite are sucrose, asparagine, alanine, raffinose,

citrate, and malate [23]. Due to the higher osmotic potential, nutrients easily move toward the parasite [24].

Following successful attachment and transfer of nutrients, the haustorium differentiates into a tubercle. *P. aegyptiaca* started to be visible as a young tubercle at 30 DAS of *B. campestris* [Figures 1A and 1B]. A significant difference was observed in the number of *Phelipanche* stages with time [Table 2]. After a while, young tubercles become large in size and represent mature tubercles followed by buds [Figures 1C–G]. Several adventitious roots started to appear on tubercles and buds. Furthermore, the shoot gets elongated and emerges from the soil surface. There were no obvious parasitism-related signs in the host plant's shoot. We were unable to find *P. aegyptiaca* without carefully excavating and studying the root systems. The duration of the subterranean stage in *P. aegyptiaca* life cycle varies between 15 and 90 days depending on its surroundings. In pot studies, the first shoot emergence occurs after 90 DAS [Figure 1J], whereas in field conditions shoot emergence has taken place after 60 DAS [Figure 2A]. As *P. aegyptiaca* grows from the ground after a long time of infestation, mostly crop damage has already occurred. Therefore, the control strategies should target the initial key stages such as seed germination, haustoria development, and tubercle formation.

Aerial shoots began to emerge above the soil's surface on the studied *B. campestris* roots at 60 DAS, and after a little while, flowers started appearing [Figures 3A–E], and then seeds commenced to develop [Figures 4A–D]. It is estimated that a whole *Phelipanche* plant may carry up to 500,000 seeds that can survive in the subsoil for up to 20 years [6]. This phenomenon was favored by the farmers' statement during the survey. They affirmed that *Brassica* crop growing in a *Phelipanche* contaminated field after many years gap also results in the large intensity of *Phelipanche* infestation. The tiny size and appearance of *P. aegyptiaca* seeds similar to soil particles are also major limitations of their management. As an outcome, we can state that *P. aegyptiaca* illustrates two main life stages: (i) the independent phase and (ii) the parasitic phase. Seed conditioning and germination are included in the independent phase, whereas stages after host–parasite attachment are categorized in the parasitic phase [Figure 5]. At the start of the growing season, *Phelipanche* seeds undergo the preconditioning phase and become sensitive to the host-secreted chemical stimulants. After seed conditioning, seeds germinate and a radicle emerges. The *Phelipanche* stages that occur between seed germination and haustorium penetration are microscopic. They cannot be detected by outside observation.

Phelipanche development is influenced by many significant abiotic factors, including temperature. The stimulation of germination factors requires changes in soil temperature during a warm, moist period. The temperature in the coldest month may have an impact on seed conditioning [25]. The high temperature during the maturation phase (fructification phase) may explain the increase in the *Phelipanche* shoot number in every plot at the end of the growing season. Previous studies have recommended that the optimum temperature for *P. aegyptiaca* seed germination is 20–26°C [26]. Therefore, late sowing of the mustard crop could be also concluded as a broomrape control practice due to low temperatures in later months. But late sowing of mustard has its consequences such as poor growth and yield reduction of crop due to low temperature and shorter period of growing season. In addition, the severity of the parasitic weed may be influenced by the texture of the soil. Broomrape species often grow on sandy soils with low nitrogen availability, and greater coarse sand composition was significantly correlated with the severity of broomrape attacks [27,28].

P. aegyptiaca was found to heavily infect fields with poor crop conditions during the survey. Crop with nutrient-rich organic soil and

proper irrigation facility reduces the growth and delayed *P. aegyptiaca* development. As plot 3 followed by plot 2 exhibited impoverished crop conditions, *Phelipanche* incidence reached 100% only in the flowering stage [Figure 6A]. *P. aegyptiaca* assimilates from the host's vascular system and serves as an additional sink when it is parasitizing. The heterotrophic mode of *P. aegyptiaca* creates plant starvation and stress conditions that ultimately lead to yield losses [29]. Host biomass was consistently decreased in the *Brassica* fields by *P. aegyptiaca* parasitism in a manner that depended on the severity of the infection. As plot 3 had the highest *Phelipanche* severity, it showed the lowest amount of fresh crops and dry matter compared to other fields.

Traits of the broomrape life cycle such as hard seed coat, which is responsible for their long life durability in soil, production of tiny seeds in huge numbers, and seed germination only in the host presence are contributing to its success. During the survey, interaction with farmers revealed that they start to avoid mustard cultivation due to heavy infestation and high production losses. It is very difficult to kill the weed without harming the host plant because of its direct connection with the host physiology. Therefore, the physical and biochemical relationship of parasitic weeds with their host is the most challenging factor that makes weed management complicated. The cultural and physical practices are not enough to reduce the infestation due to the huge seed bank in the soil. The present investigation allowed us to rethink the known control strategies of the parasite *P. aegyptiaca* in an effective manner.

5. CONCLUSION

A combination of field and pot experiments has been performed to evaluate the different stages of the *P. aegyptiaca* life cycle. *P. aegyptiaca* passes through several developmental phases, including the formation of radicles, tubercles, buds, subterranean and aerial shoots, flowering, and seed production. From the obtained results, we can conclude that all *P. aegyptiaca* ontogenetic phases appeared between 15 and 90 days after *B. campestris* seeds were planted. From the germination of seeds to the production of seeds, the entire life cycle takes 4–5 months. The present study also revealed the effect of *P. aegyptiaca* infestation on the biomass of *B. campestris* and its distribution in several regions of northeastern Rajasthan. Future research can use the results of this study to design efficient control methods that would especially avoid the damage it causes in agricultural regions.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

9. CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest or close personal connections that may have appeared to affect the research presented in this study.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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