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## Allelopathic potential of purple nutsedge (*Cyperus rotundus* L.) on germination and initial seedling vigour of Bt cotton hybrid

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### Abstract

Allelopathic effects of *Cyperus* spp. have been generally reported and are considered the major factor limiting the initial establishment of agricultural crops. A laboratory study was conducted to examine the allelopathic effects of *Cyperus rotundus* L. on Bt cotton. Since it was hypothesised that *C. rotundus* exudates enters in the rhizosphere environment in the form of aqueous extract at the time of germination of tubers. The allelopathic effect was assessed in series of laboratory environments at Department of Agronomy, Annamalai University, Annamalai Nagar, Tamil Nadu during start of two seasons viz., October 2023 (Rainfed) and January 2024 (Irrigated). At the time of cropping the germinated *C. rotundus* rhizomes were collected from the cotton field and the aqueous stock solution of 1:1 (w/v) was prepared and dilutions were made in 0, 10, 20, 30, 40 and 50% concentrations. Bt cotton seeds (RCH 659 BG II) were incubated for 10 days under respective concentrations and biometrics were recorded. The pooled data of the present experiment indicated that aqueous extract of germinated rhizomes *C. rotundus* significantly reduced the frequency of percent germination, seed germination potential; increased the percent mortality rate, abnormal germination rate, germinal length inhibition index and seedling vigour index.

**Keywords:** Allelopathy, germination inhibition, hybrid cotton, phenolic acid, vigour index, weeds

### Introduction

Weeds are unwanted, troublesome, noneconomic and harmful vegetations that compete with associate plants for light, water, space, nutrients, and thus cause significant loss to global agricultural production systems (Singh *et al.*, 2022) [24]. It has been reported that weeds cause yield reduction up to 10-100%, depending upon crop and related weed flora, weed density, time of competition, duration of competition, management practices and agro-climatic situations (Damalas and Koutroubas *et al.*, 2022) [6]. The early adaptative nature and rapid growth of weed make it able to impede the desired crop growth and diminish the yields (Travlos *et al.*, 2020) [29].

The interference of weeds with crops may be the consequence of competition and/or allelopathy. Allelopathy is prevalent in agricultural ecosystems and mediated by plant-derived secondary metabolites (allelochemicals). Allelochemicals are released by donor plants and affect the root growth and development of receptor plants (Huang *et al.*, 2020) [13]. The release of specific Allelo-chemicals from leaves, root, rhizome and stem into the surrounding growing environment between weeds and agricultural plants can have both inhibitory and stimulatory effects. The allelopathic effect of different parts of a single weed plant also varies in their influence on crops seed germination and development (Shah *et al.*, 2022) [22].

*Cyperus rotundus* L., (Purple nutsedge) belonging to the family Cyperaceae, native to India, is the world's worst noxious weed of different economically important crops. Its rapid growth tendency and extensive underground rhizome makes it extremely difficult to control. Although this weed also produces seeds but it mostly spreads by tubers (Zoheir *et al.*, 2009) [30]. This weed has been reported to cause 20–90% yield losses in various crops across the world (Peerzada 2017) [18].

Perennial nature, genetic diversity, high rate of reproduction, ability to tolerate adverse climatic conditions, ease-of-dispersion, strong competitive abilities, and allelopathic potential assist this weed to thrive in a wide range of agro-climatic regions (Travlos *et al.*, 2020) [29]. Due to dormancy, the tubers also sustain in the soil for longer time and interfere with the crops raised in the succeeding season.

Cotton (*Gossypium hirsutum* L.) is a major textile industry crop and in 2022-23 India's acreage under cotton was 13,061 lakh hectares (33% of the world's total cotton acreage), the highest in the world. Bt cotton varieties dominate hybrid cotton production in India however the production has dipped to 15-year low due to multiple factors. India (5.7 million metric tons) being the second largest producer in the world after China (6.7 million metric tons) (Shahbandeh, 2023) [23]. The productivity per hectare during 2022 growth season was 445 kgs, which was significantly lower in comparison with the top producer (2,015 Kgs per ha) and world (662 Kgs per ha) average (Sandhya, 2023 & Textor, 2024) [20, 28].

Heavy weed infestation is one of the major low per-hectare production of cotton. Cotton is much more vulnerable to weed competition due to the broad spacing requirement between the plants (Chen *et al.*, 2022) [4]. Presence of allelochemicals of certain weeds such as *C. rotundus* also inhibit or delayed germination of cotton seeds which may reduce the vigour index of seedlings, and ultimately suppress the growth and productivity. Although the economic damage caused by *C. rotundus* production of various agriculture crops, there are no reports of allelopathic effects of these species on seeds germination of hybrid Bt cotton. Therefore, this experiment was aimed to evaluate the allelopathic potential of aqueous extracts of *C. rotundus* germinated rhizome on hybrid Bt cotton seeds germination.

## Materials and Methods

The present study was carried out at the Agronomy Experimental laboratory (11°24' North latitude, 79°44' East longitude and at an altitude of +5.79 msl), Annamalai University, Annamalai Nagar, Tamil Nadu, during the start of two cropping seasons *viz.*, October 2023 (Rainfed) and January 2024 (Irrigated). The weather is moderately warm with hot summer months with mean annual rainfall of 1550 mm. About 80% of rainfall is distributed during North East monsoon (Oct-Dec). The mean maximum temperature is 24.3 during monsoon while 34.8 °C during summer with the average RH of 78%.

The germinated rhizomes were collected from the *C. rotundus* severely infected cotton fields at the time of germination of Bt cotton crop. Immediately after collection rhizomes were thoroughly washed to remove the soil particles. The aqueous extract of 100% concentration was prepared by crushing fresh germinated tubers by immersing them in distilled water for 24 hours with a 1:1 weight/volume ratio, and filtered using Whatman no 1 filter paper. Then diluted with distilled water to achieve desired concentrations of 10%, 20%, 30%, 40% and 50% extract.

The seeds of hybrid Bt cotton (RCH 659 BG II) were surface sterilized with 1.0% sodium hypochlorite solution for one minutes, then rinsing them twice with sterile water and blotted dry. The seeds were soaked for overnight in different concentration of respective rhizome extracts and seeds soaked in distilled water as control. Total of five treatments (10%, 20%, 30%, 40% and 50%) and a control each replicated four times were placed in randomized complete block design.

Three layers of filter paper (Whatman No. 1) were placed in a

petri-dish, moisture with respective concentrations and 20 numbers of seeds were placed. These experimental set up was placed in Laboratory at a room temperature (27°C) and relative humidity of 72%. Each petri-dish was moistened with 5 ml of respective concentrations of extract each time, when necessary. The changes in the seed germination were recorded daily. The seeds were considered to have sprouted when the tip of the radicle appeared to have free of seed coat. The germination period was determined as the number of days from the first observed germination to the time when there was no germination.

After ten days of incubation, five seedlings were randomly selected from each petri-dish, and the required parameters were analysed. The first count was obtained by the percentage of normal seedlings obtained at fifth day. The final germination percentage was obtained by counting the normal seedlings observed after 10 days after treatment, it was considered normal for the seedlings that had radicle greater than 5 mm in length and shoot with leaflets expanded. The germination analysis at the first count is significant to determine the allelopathic effect. The germination indices were analysed by using the following equations. Germination percent was calculated using the equation suggested by Scott *et al.*, (1984) [21] and expressed as percentage.

$$G\% = \frac{Gn}{N} \times 100$$

Where,  $Gn$  is the number of seeds germinated 10 days after treatment and  $N$  is the total number of seeds sown.

Seed germination potential was calculated using the equation suggested by Pan *et al.*, (2023) [17] and expressed as percentage.

$$Gp\% = \frac{NGn}{N} \times 100$$

Where,  $NGn$  is the normal germination number and  $N$  is the total seed number within 5 days of germination.

Mean germination time is the average time to germination or for the radicle to reach 2 mm. Mean germination time was calculated by using the equation of Ellis and Roberts (1980) [8] and expressed as days.

$$MGT (days) = \frac{\sum_{i=1}^k ni \cdot ti}{\sum_{i=1}^k ni}$$

Where,  $t_i$  is the time (days) from the start of the experiment to the  $i$ th observation,  $n_i$  is the number of seeds germinated at the  $i$ th observation and  $k$  is the day of last observation.

Germination rate was evaluated by the equation proposed by Maguire (1962) [16]. The germination rate is calculated by dividing the number of normal seedlings obtained at each counting by the number of days seeds have been incubated. The values obtained at each count are then summed at the end of the germination test to obtain the germination rate:

$$Gr = \frac{\text{number of normal seedlings}}{\text{days to first count}} + \dots + \frac{\text{number of normal seedlings}}{\text{days to final count}}$$

Percent mortality rate is calculated by dividing the number of seeds that don't germinate by the total number of seeds tested, and then multiplying by 100 and expressed as a percentage.

Percent mortality rate was evaluated by using the following equation.

$$MR = \frac{MRn}{GN} \times 100$$

Where, *MR* is percentage mortality rate, *MRn* is the number of seeds perished after germination and *GN* is the total number of seeds germinated.

Abnormal germination rate was evaluated by using the following equation

$$AGr\% = \frac{AGs}{N} \times 100$$

Where, Abnormal germination rate (%), *AGs* - Number of abnormal germinated seeds and *N* - Total number of seeds germinated.

The germinal length inhibition index (GII) is a measurement used to assess the impact of a treatment on the root or shoot growth of germinating seeds compared to a control group. Germinal length inhibition index was evaluated by using the following equation:

$$GSI = \frac{Cgl - Tgl}{Cgl} \times 100$$

Where, *Gli* - Germinal length inhibition index (%), *Cgl* - Control germinal length and *Tgl* - Treatment germinal length

The root shoot ratio on the basis of seedling length was calculated by using the following equation.

$$\text{Root Shoot Ratio (Seedling Length basis)} = \frac{\text{Root Length}}{\text{Shoot Length}}$$

The vigour level of treated seeds was calculated by using the procedure suggested by Abdul-Baki and Anderson (1973) [1]. Seed Vigour Index<sub>(Mass)</sub> in terms of mass was determined by multiplication of germination percentage with seedling dry weight.

$$\text{Seedling vigour index (Mass)} = \text{Germination \%} \times \text{AverageSeedling Dry Weight (mg)}$$

### Statistical analysis

Seed germination and initial growth seedling experiments utilized a completely randomized design with four replications. The angular transformation of  $\text{Arc sin } \sqrt{x}$  was used for normalizing of the data. The results obtained in seeds germination of each species were subjected to polynomial regression using the SPSS Version 24 statistical program.

### Results

Germination percent indicates the proportion of seeds successfully sprout under specific treatments. The effect of *C. rotundus* rhizome extract concentrations on germination percentage was exhibited the strong coefficient of determination ( $R^2=0.9693$ ). The germination inhibition was decreased from 92.25% in 10% concentration to 31% in 50% concentration (Fig. 1). Seed germination potential indicates the likelihood of a seed successfully germinating and developing into a seedling under ideal conditions. The *C. rotundus* rhizome extract concentrations was exhibited the robust coefficient of determination ( $R^2=0.9772$ ) on the seed germination potential. Seed germination potential in the control was 55.5% and it was strongly reduces from 52.25% in 20% concentration to 7% in 50% concentration (Fig. 2).

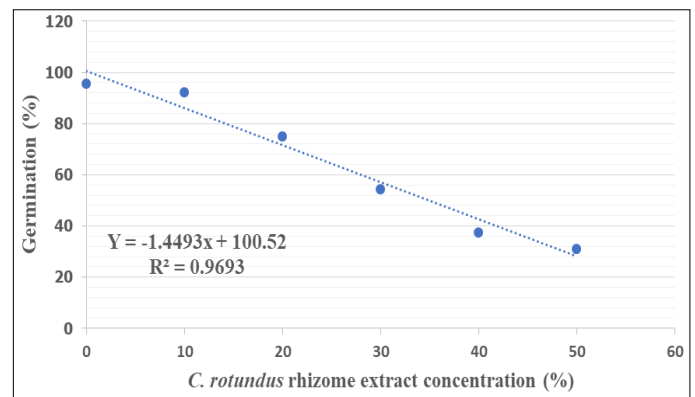


Fig 1: Germination percentage of aqueous rhizome extracts of *C. rotundus* on Hybrid Bt cotton

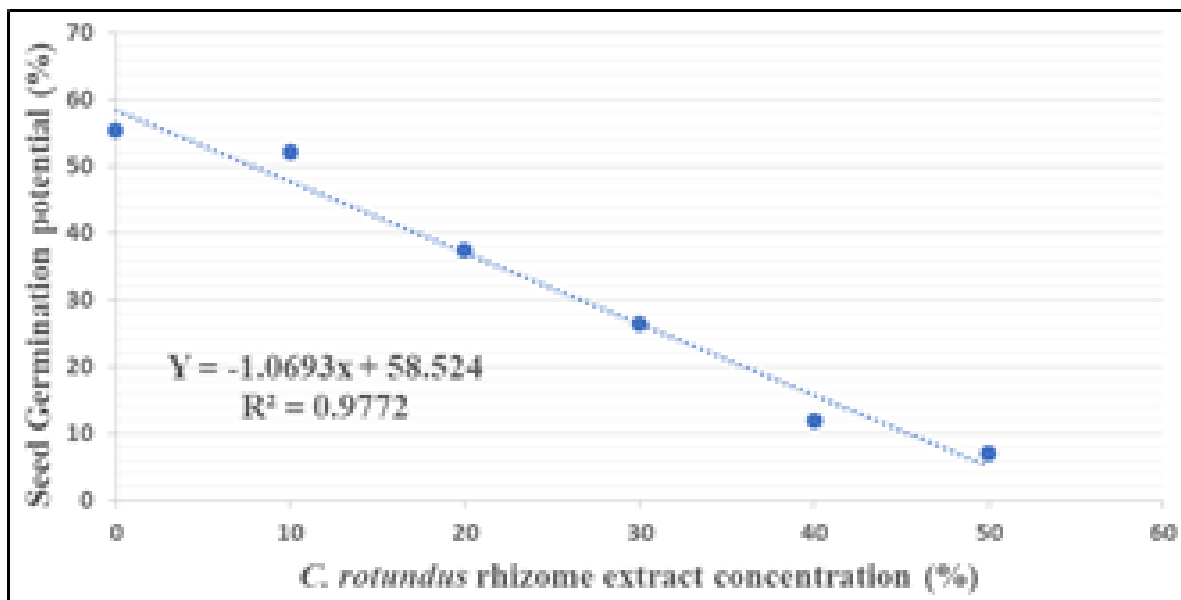
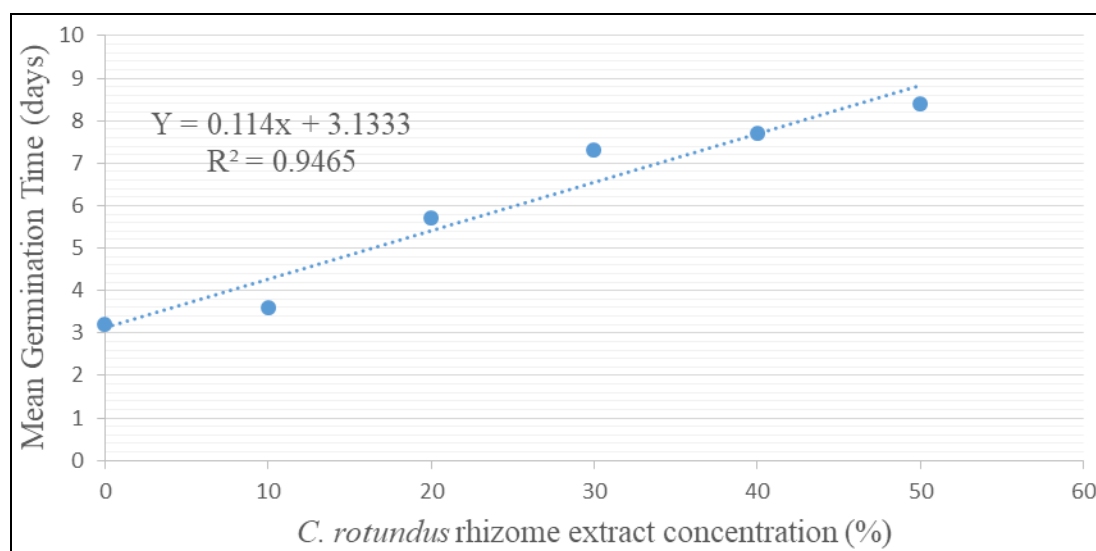


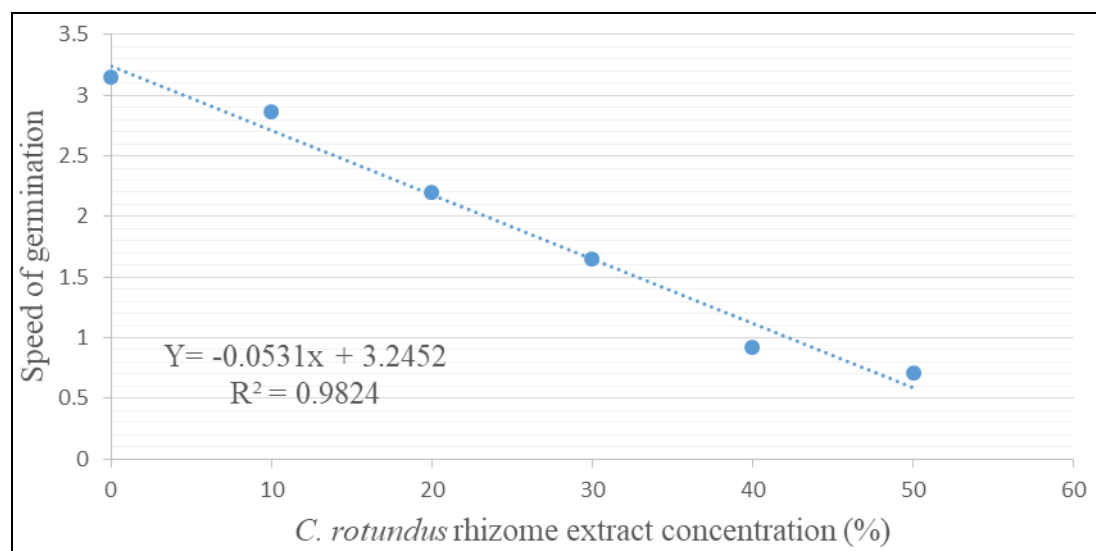
Fig 2: Seed germination potential (%) of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

Mean germination time is used to assess the average time it takes for a seed population to germinate. It serves as an indicator of seed vigor, which reflects the overall health and sprouting potential of the seeds. The consequence of *C. rotundus* rhizome extract concentrations on mean germination time was registered the strong coefficient of determination ( $R^2=0.9465$ ). The mean germination time increases when treatment concentration increases. The control exhibited the mean germination time of 3.2 days while the increment in the treatment was from 5.7 days in 20% concentration to 8.4 days in 50% concentration (Fig. 3). Speed of germination measures the time taken by individual seeds within those specified seeds germinated. A high speed of

germination value indicated that a larger portion of the seeds are germinating within a relatively short time frame. Earlier emergence of seedlings allows them to establish themselves before facing competition from weeds and also harsh weather conditions. The *C. rotundus* rhizome extract concentrations was registered the strong coefficient of determination ( $R^2=0.9824$ ) on speed of germination. It decreases when treatment concentration increases. The control exhibited the mean speed of germination value of 3.15 while the decrease in the treatments were from 52.20 in 20% concentration to 0.71 in 50% concentration (Fig. 4).



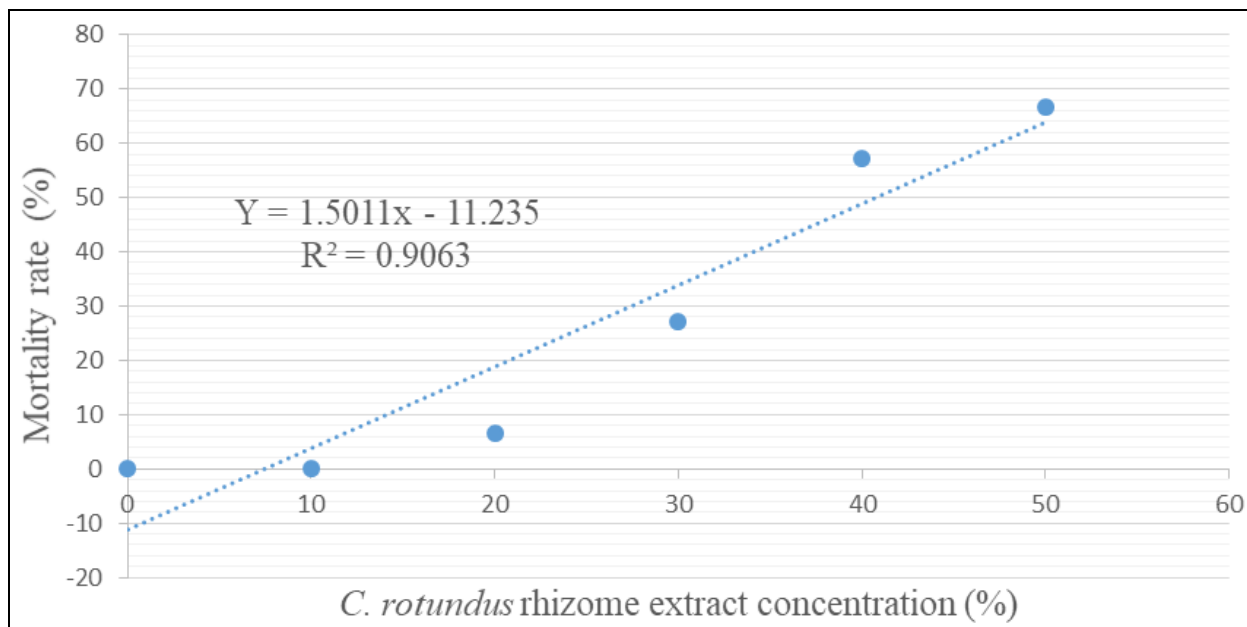
**Fig 3:** Mean germination time (days) of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*



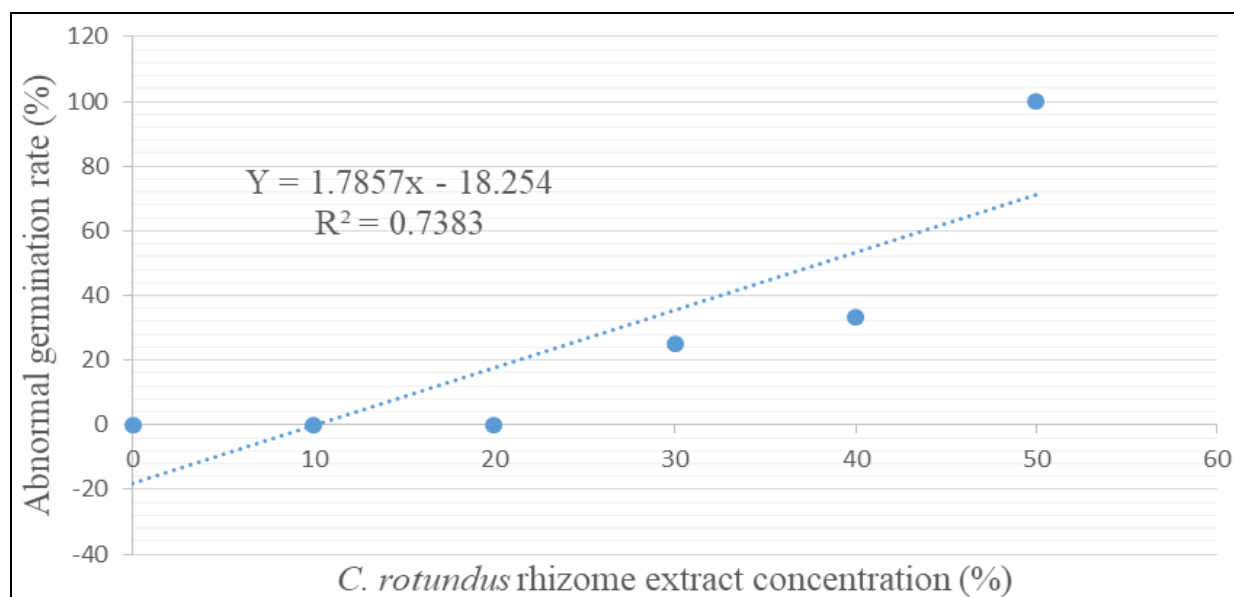
**Fig 4:** Speed of germination of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

Percent mortality rate indicates the percentage of seeds within a group that fail to germinate and establish themselves as seedlings. A lower seed mortality rate indicates a higher success rate in seed germination and seedling establishment. The different concentrations of *C. rotundus* rhizome extract was

recorded the strong coefficient of determination ( $R^2=0.9063$ ) on percent mortality rate. It increases when treatment concentration increases. There was no percent mortality rate in control and 10 percent concentration while it was higher in 30% (27.27%), 40% (57.14%) and 50% (66.67%) concentration (Fig. 5).



**Fig 5:** Mortality rate of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

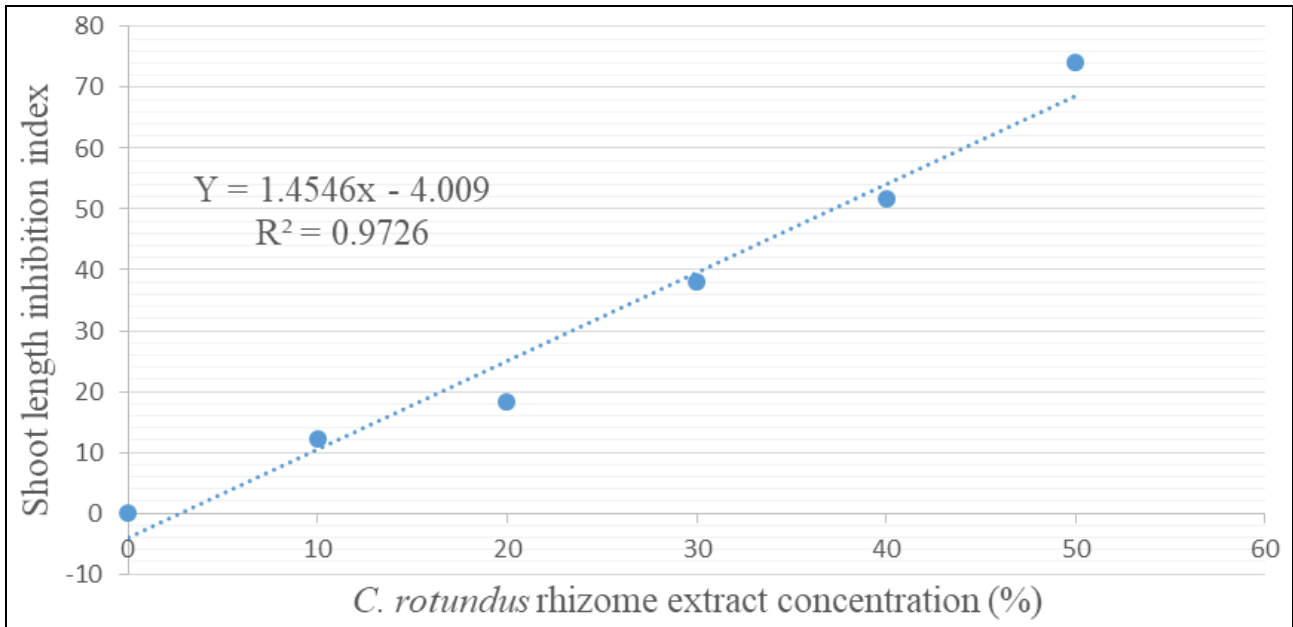


**Fig 6:** Abnormal germination rate (%) of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

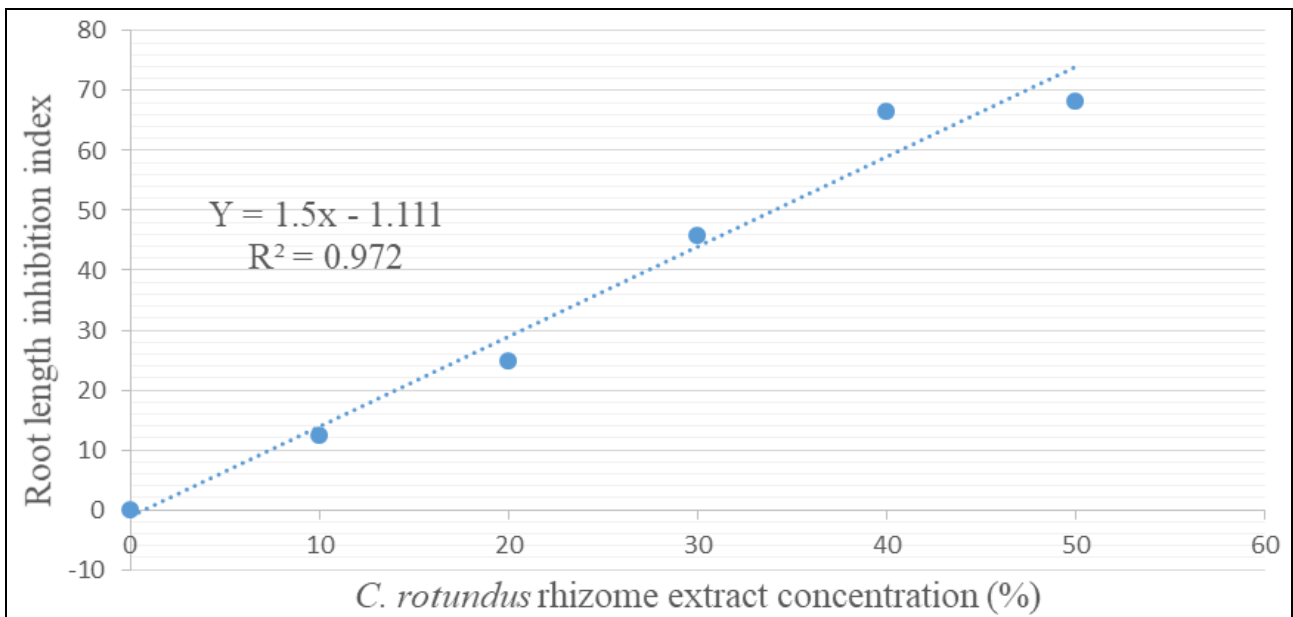
Abnormal germination rate refers to the percentage of seeds within a group that sprout but exhibit some kind of deformity or weakness that hinders their ability to develop into healthy, mature plants. The *C. rotundus* rhizome extract concentrations registered the strong coefficient of determination ( $R^2=0.7383$ ) on abnormal germination rate. It increases from 30% concentration onwards. There was no Abnormal germination rate up to 30 percent concentration while at 50% concentration it exhibited the 100% abnormal germination rate (Fig. 6).

The germinal length inhibition index (GII) quantifies the

reduction in root or shoot length caused by a particular treatment applied to seeds during germination. A higher GII value indicates a greater inhibitory effect on root or shoot growth. The different concentrations of *C. rotundus* rhizome extract registered the strong coefficient of determination ( $R^2=0.9726$ ) on shoot length inhibition index (GIIshoot) of Bt hybrid cotton. The GIIshoot value was 0 for control and it significantly increases when treatment concentration increases. The increment was from 16.13% in 10% concentration to 74.19% in 50% concentration (Fig. 7).



**Fig 7:** Shoot length inhibition index (GIIshoot) of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

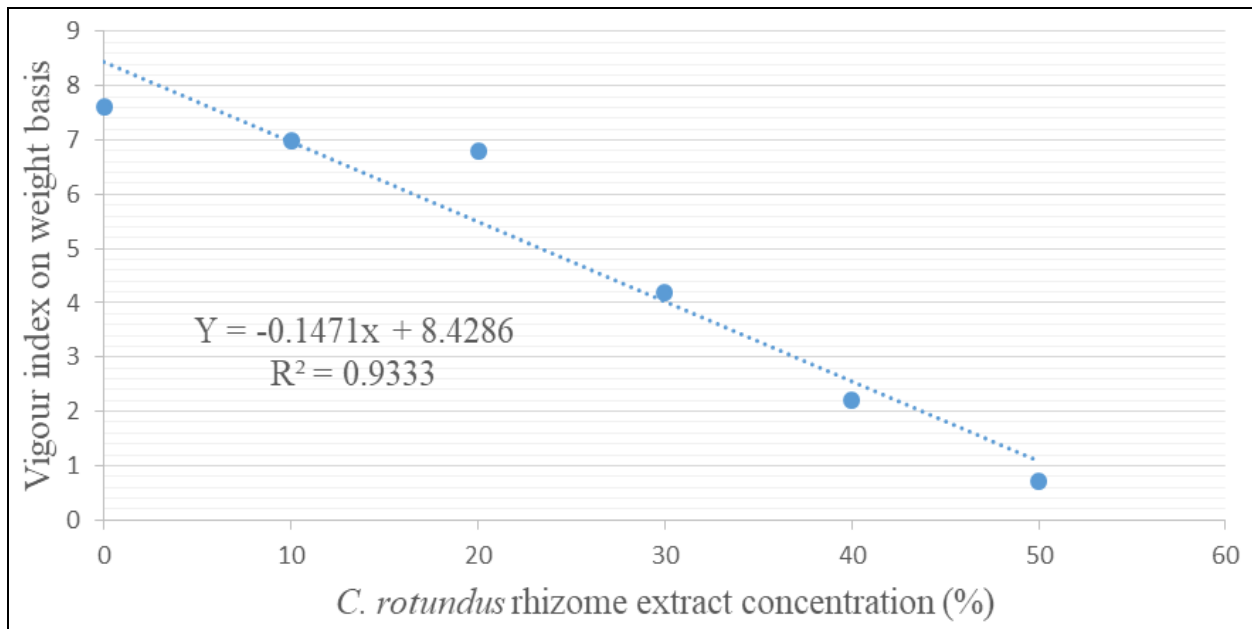


**Fig 8:** Root length inhibition index (GIIroot) of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

Likewise, the  $GII_{root}$  value was 0 for control and it significantly rises when treatment concentration increases. The increment was from 12.5% in 10% concentration to 83.33% in 50% concentration (Fig. 8). A  $GII$  close to 0% suggests minimal inhibition by the treatment, while a  $GII$  of >80% signifies that the treatment reduced root or shoot length by 80 percent compared to the control group.

The vigor index of seedlings on a weight basis is a metric used to assess the overall health and establishment potential of germinated seeds. A higher vigor index indicates a healthier and

more vigorous seedling population. These seedlings have a higher chance of successful establishment and growth into mature plants. The *C. rotundus* rhizome extract concentrations was registered the strong coefficient of determination ( $R^2=0.9333$ ) on vigor index of Bt cotton seedlings. It decreases when treatment concentration increases. The sturdy decrease in the vigor index was observed from 30% concentration onwards (4.2) and 50% concentration registered the vigor index value of 0.7 (Fig. 9).



**Fig 9:** Vigour index (on weight basis) of Hybrid Bt cotton at different concentrations of aqueous rhizome extracts of *C. rotundus*

### Discussion

Germinating rhizome extract of *Cyperus rotundus* severely affected the germination and early growth of Bt hybrid cotton in a dose-dependent manner. The inhibitory effect of rhizome extract was more pronounced on 30% concentration onwards. It significantly decreased germination percentage, seed germination potential, speed of germination, while increased mean germination time, mortality rate, abnormal germination rate, germinal length inhibition index and ultimately decreased the seedling vigour. Several reports revealed that the inhibitory effect of *Cyperus rotundus* is due to allelopathic effect (Ameena *et al.*, 2015; Dhima *et al.*, 2016) [2,7]. Various secondary metabolites produced by plants and micro-organisms have been considered as potential allelochemicals. They are released by a donor plant into the environment *via* volatilization, leaching, root exudation, or decomposition of plant residues, which is the greatest source of allelotoxins (Staszek *et al.*, 2021) [27].

In agroecosystems, allelochemicals have detrimental effects on the growth of associated and or next-season crops. In addition, weeds can exhibit allelopathy against crop plants. The strong allelopathic effects exhibited by plants play a significant role in promoting their invasion by altering the dynamics and interactions within the invaded ecosystem (Pan *et al.*, 2023) [17]. Phenolic acids constitute a vital group of allelochemicals that release into the soil or rhizosphere environment from plants through numerous mechanisms including root exudation (Rice, 1974). Upon release, they play a multitude of ecological and physiological roles *viz.*, inhibit plant growth, disrupt membrane permeability, induce water stress, affect photosynthesis and protein synthesis, alter enzyme activities, *etc.* The water extract of *C. rotundus* tuber contains phenolic acids such as Ferulic acid (35.8%), Vanilic acid (33.67%) and Caffieic acid (21.73%) (El-Rokiek *et al.*, 2010) [9].

Among them Ferulic acid and Caffieic acid have exhibits strong allelopathic effect on plants. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is produced by plants and subsequently released into soils involved in biochemical interactions between plants (allelopathy). Ferulic acid inhibits seed germination, root and shoot growth, cell division, and dry weight accumulation and induces several physiological alterations in the plants (Blum and Dalton, 1985) [3]. Ferulic acid inhibits photosynthesis,

reduces leaf chlorophyll content, disrupts ion and water uptake, and plant water utilization, cause stomatal closure by reducing turgor and osmotic pressure and inhibits foliar expansion.

Ferulic acid inhibits root growth and development, and *in vitro* rooting process in mung bean by interfering with biochemical processes that are crucial for root formation (Singh *et al.*, 2014) [25]. Ferulic acid caused a reduction in the contents of water-soluble proteins and endogenous total phenolics, whereas the activities of proteases, peroxidases, and polyphenol peroxidases increased. Even low concentrations of ferulic acid significantly inhibited stomatal opening, the stomatal opening ratio, stomatal length and width and inhibited net photosynthetic rate (Fu *et al.*, 2019) [10].

Pan *et al.*, (2023) [17] observed that caffeic acid did not significantly affect root length during seed germination. However, it significantly inhibited root growth at higher concentrations during the subsequent plant growth stage. Caffeic acid significantly suppressed root growth of mung bean, and impaired adventitious root formation and root length. It also interference with the biochemical processes involved in rooting process such as root initiation, root expression, and post-expression of rhizogenesis (Daizy *et al.*, 2008) [5].

Likewise, reactive oxygen species (ROS) have significant roles in plant physiology, and regulate radicle emergence and root elongation in a non-enzymatic manner during dicot seed germination (Li *et al.*, 2017; Huang *et al.*, 2020) [14,12]. However, their imbalance results in disruption of cell structure and deregulation of cellular processes. Intense allelopathic exposure can lead to over production and accumulation of ROS in the plants (Šoln *et al.*, 2022) [26]. At the cellular level, allelochemicals induce a burst of ROS, which leads to oxidative stress, and can promote cell death (He *et al.*, 2022) [11].

Lipid peroxidation and cell membrane changes, protein modifications, and increased protease activities are the early signs of cell damage (Liu *et al.*, 2018). When enzymatic and nonenzymatic antioxidants cannot scavenge reactive oxidants, this can result in hydrolytic or necrotic degradation of the protoplast. Cell organelles then lose their integrity and function. In roots, the structure and activity of the apical meristem are changed, which affects root growth and water absorption and ultimately development of plants. these are all influence the

germination of Bt hybrid cotton. The accumulation of these phenolic acids could create a more favourable environment for *C. rotundus* to compete for growth space and resources and promoting their further colonization and inhibit the growth of economically important crops including hybrid cotton.

### Conclusion

The present study concluded that at higher concentrations (>30%), *C. rotundus* rhizome extract significantly decreased germination percentage, seed germination potential, speed of germination, while increased mean germination time, mortality rate, abnormal germination rate and germinal length inhibition index. The inhibitory effect was more pronounced due to the presence of phenolic compounds such as ferulic acid and caffeic acid. The *C. rotundus* heavily infested fields are fail to exhibit initial vigour of crops such as Hybrid Bt cotton and reduced the chance of healthier and more vigorous seedling population. This have a less chance of successful establishment and growth into mature plants unless the efficient weed control methods are not followed.

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